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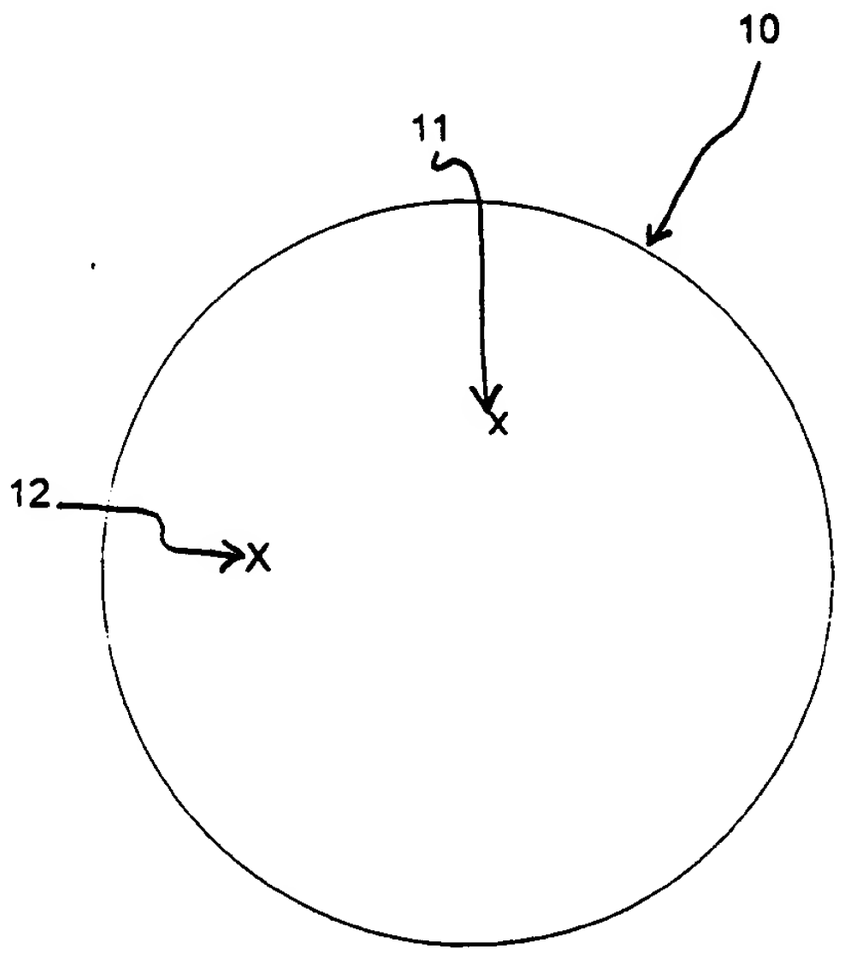
(57) Abstract

A microsphere–based analytic chemistry system is disclosed in which self–encoding microspheres having distinct characteristic optical response signatures to specific target analytes may be mixed together while the ability is retained to identify the sensor type and location of each sensor in a random dispersion of large numbrs of such sensors in a sensor array using an optically interrogatable encoding scheme. An optical fiber bundle sensor is also disclosed in which individual microsphere sensors are disposed in microwells at a distal end of the fiber bundle and are optically coupled to discrete fibers or groups of fibers within the bundle. The identifies of the individual sensors in the array are self–encoded by exposing the array to a reference analyte while illuminating the array with excitation light energy. A single sensor array may carry thousands of discrete sensing elements whose combined signal provides for substantial improvements in sensor detection limits, response times and signal–to–noise ratios.



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SELF-ENCODING FIBER OPTIC SENSOR

FIELD OF THE INVENTION

The present invention is generally concerned with chemical sensors, sensor arrays and sensing apparatus for the detection of gaseous and liquid analytes. More particularly, the invention is directed to optical chemical sensors and the detection and evaluation of optical data generated by sensing receptor units.

BACKGROUND OF THE INVENTION

The use of optical fibers and optical fiber strands in combination with light absorbing dyes for chemical analytical determinations has undergone rapid development, particularly within the last decade. The use of optical fibers for such purposes and techniques is described by Milanovich et al., "Novel Optical Fiber Techniques For Medical Application", Proceedings of the SPIE 28th Annual International Technical Symposium On Optics and Electro-Optics, Volume 494, 1980; Seitz, W.R., "Chemical Sensors Based On Immobilized Indicators and Fiber Optics" in *C.R.C. Critical Reviews In Analytical Chemistry*, Vol. 19, 1988, pp. 135-173; Wolfbeis, O.S., "Fiber Optical Fluorosensors In Analytical Chemistry" in *Molecular Luminescence Spectroscopy, Methods and Applications* (S. G. Schulman, editor), Wiley & Sons, New York (1988); Angel, S.M., *Spectroscopy* 2(4):38 (1987); Walt, et al., "Chemical Sensors and Microinstrumentation", *ACS Symposium Series*, Vol. 403, 1989, p. 252, and Wolfbeis, O.S., *Fiber Optic Chemical Sensors*, Ed. CRC Press, Boca Raton, FL, 1991, 2nd Volume.

When using an optical fiber in an *in vitro/in vivo* sensor, one or more light absorbing dyes are located near its distal end. Typically, light from an appropriate source is used to illuminate the dyes through the fiber's proximal end. The light propagates along the length of the optical fiber; and a portion of this propagated light exits the distal end and is absorbed by the dyes. The light absorbing dye may or may not be immobilized; may or may not be directly attached to the optical fiber itself; may or may not be suspended in a fluid sample containing one or more analytes of interest; and may or may not be retainable for subsequent use in a second optical determination. Once the light has been absorbed by the dye, some light of varying wavelength and intensity returns, conveyed through either the same fiber or collection fiber(s) to a detection system where it is observed and measured. The interactions between the light conveyed by the optical fiber and the properties of the light absorbing dye provide an optical basis for both qualitative and quantitative determinations.

More recently, fiber optic sensors have been constructed that permit the use of multiple dyes with a single, discrete fiber optic bundle. U.S. Pat. Nos. 5,244,636 and 5,250,264 to Walt, *et al.* disclose systems for affixing multiple, different dyes on the distal end of the bundle, the teachings of each of these patents being incorporated herein by this reference. The disclosed configurations enable separate optical fibers of the bundle to optically access individual dyes. This avoids the problem of deconvolving the separate signals in the returning light from each dye, which arises when the signals from two or more dyes are combined, each dye being sensitive to a different analyte, and there is significant overlap in the dyes' emission spectra.

Most recently, fiber optic sensors have been employed in arrays of semi-selective chemical sensors and pattern recognition schemes to discriminate and quantify odors. Such approaches have been useful in implementing the principles of biological olfaction in the design of sensing devices or systems. In this field of biomimetry, various technologies have been applied to the sensor transduction mechanism. For example, surface acoustic wave, conducting polymer, metal oxide sensor field-effect transistor (MOSFET), piezo-electric, and quartz crystal microbalance sensor arrays have been pursued.

While such technologies provide inventive approaches utilizing a variety of physical and chemical phenomena to odor sensing, there are a number of limitations to these methods which restrict the efficacy of such devices. Firstly, element-to-element reproducibility both within a single array and between sensor arrays is typically unsatisfactory and thus requires recalibration and network retraining from sensor to sensor. Secondly, most of these methods have a relatively slow response time, frequently requiring several minutes to respond to the presence of an odor. Thirdly, such methods have relatively high detection limits and low sensitivity, typically not functioning at odor levels below 10ppm. Fourthly, devices which embody such technologies typically require a relatively large inherent size, thereby restricting miniaturization of the sensor array for use in remote sensing applications. Finally, construction of multi-sensor arrays by these methods is complex and involves expensive and tedious preparation and placement of individual sensors within a well-defined array.

Most recently, many of these shortcomings have been overcome through the application of fiber optic sensor arrays in an artificial nose sensor device and system. U.S. Pat. Nos. 5,320,814 and 5,512,490 to Walt,et al., the teachings of each of these patents being incorporated herein by reference, disclose a fiber optic array formed of heterogeneous, semi-selective thin films which function as sensing receptor units and are able to detect a variety of different analytes and ligands using spectral recognition patterns. This technology has been applied to a vapor-sensing system which utilizes arrays of polymer-dye combinations which coat the ends of select optical fibers in a fiber optic bundle. These developments are further described in Dickinson, et al, *Nature* 382:697 (1996) and White, et al, *Anal. Chem.* 68:2191 (1996).

encoding dye choice is further limited by selecting dyes whose emission wavelengths do not overlap or interfere with the reporting dye which is uniquely responsive to the presence of an analyte.

Another limiting feature of this invention is that the process of encoding beads requires a series of measurements which calibrate and train the sensors and the sensor array. Encoding is initially accomplished by first illuminating the beads with excitation light energy and monitoring and recording the type and location of the specific bead subpopulation within the sensor array having a given encoding dye ratio. Next, the array is exposed to an analyte while illuminating the array with excitation light energy in the presence of a reporter dye. Those beads which are responsive to the analyte in the presence of the reporter dye are monitored and mapped on the sensor array. In addition, the characteristic optical response signature is stored in a library. This step is repeated for each analyte of interest in combination with a reporter dye. Once all bead subpopulations are encoded and their response characteristics monitored and recorded, the entire sensor array must be decoded for each analyte by indexing each sensor element with the stored optical response signature for each analyte. This process of decoding individual subpopulations of beads may be require additional steps when a large number of subpopulations are deployed in the array, thereby increasing the training time required for each array.

Other alternative approaches to bead encoding, utilizing molecular tagging, capillary gas chromatography and electron capture detection have been disclosed by Still, et al, *Acc. Chem. Res.* 29:155 (1996). However, such methods are limited in scope and have been applied only to a narrow class of bead materials having specific chemical functionality and molecular tags which are readily analyzable.

SUMMARY OF THE INVENTION

In general, the invention provides self-encoding analytic chemical sensor arrays comprising a substrate with a surface comprising discrete sites and a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises at least one reporter dye. The reporting dye has a first characteristic optical response signature when subjected to excitation light energy in the presence of a reference analyte, and the microspheres are distributed on the surface. The beads may further comprise a bioactive agent or, alternatively, a chemical functionality which interacts, associates, or binds with analytes to be detected.

In an additional aspect, the invention provides methods of detecting a target analyte in a sample comprising contacting the sample with an sensor array. The sensor array comprises a substrate with a surface comprising discrete sites and a population of microspheres. The microspheres comprise at least a first and a second subpopulation, each subpopulation comprising a bioactive agent and at least one reporter dye. The reporting dye has a first characteristic optical response signature when

Fig. 1 is a schematic diagram illustrating the self-encoding microsphere sensor according to the present invention;

Fig. 2 is a process flow diagram of the preparation, encoding and incorporation of microspheres into a sensor array of the present invention;

Figs. 3A and 3B is a schematic process diagram illustrating the preparation and placement of self-encoded microsphere subpopulations in fiber optic sensor array of the present invention;

Fig. 4 is a process flow diagram illustrating microwell formation in the fiber optic bundle and placement of the microspheres in the microwells according to the method of the present invention;

Figs. 5A and 5B are micrographs illustrating the microwells formed on the distal end of a fiber optic bundle and microspheres inserted in the microwell cavities;

Figs. 6A and 6B are micrographs showing the array of microspheres in their corresponding microwells prior to and subsequent to agitation by tapping and an air pulse, demonstrating the electrostatic binding of the microspheres in the microwell cavities;

Fig. 7 is a schematic diagram of the inventive fiber optic sensor and associated instrumentation and control system;

Fig. 8 is a schematic diagram illustrating the experimental apparatus used in the optical measurements of Examples 7 through 17;

Fig. 9 illustrates the characteristic optical response signature of porous silica beads infiltrated with Nile Red dye upon exposure to toluene vapor;

Fig. 10 illustrates the characteristic optical response signature of PMS beads infiltrated with Nile Red dye upon exposure to methanol vapor;

Figs. 11A and 11B illustrate the characteristic optical response signature of a PS802 coated porous silica bead infiltrated with Nile Red dye upon exposure to toluene and methanol vapor;

Figs. 12A and 12B illustrate the characteristic optical response signature of a PDPO coated porous silica beads infiltrated with Nile Red dye upon exposure to toluene and methanol vapor;

Fig. 13 illustrates the characteristic optical response signature of porous silica beads infiltrated with Nile Red dye upon exposure to ethyl acetate vapor;

one or more reporter dyes that exhibit a characteristic, i.e. unique, optical response signature to a reference analyte, generally a fluid such as a vapor. Thus, in this embodiment, exposure of the entire array to a reference analyte will allow the identification of the location of each bead of each subpopulation. As a result, by comparing the response of the entire sensor array to a known analyte, the individual sensor elements of the array are conveniently decoded simultaneous in one simple measurement. The self-encoding feature of the present invention eliminates the need for a more complex, multi-step encoding system.

The sensor array can then be used to detect the presence of target analytes, for example when the beads also comprise bioactive agents such as oligonucleotides or, alternatively, a chemical functional group, by looking for changes in the optical signature of the beads upon interaction or binding with a the target analyte, for example a substantially complementary labeled oligonucleotide, or, alternatively, a target analyte of interest. As will be appreciated by those in the art, this may be done in a variety of ways, generally through the use of a change in an optical signal. This change can occur via many different mechanisms. A few examples include the binding of a dye-tagged analyte to the bead, the production of a dye species on or near the beads, the destruction of an existing dye species, a change in the optical signature upon analyte interaction with dye on bead, or any other optical interrogatable event. Thus, once the location of each species of an oligonucleotide probe, or alternatively, a bead having a particular chemical functionality, has been identified, the array can then be used to detect the presence of unknowns that will preferably specifically associate with the bioactive agents or chemical functionality on the beads.

In an alternate preferred embodiment, when the target analyte is not labeled, the optical response of each element in the array can be compared to a library of characteristic optical response signatures for its corresponding bead subpopulation type, where the characteristic optical response signature to various analytes has been previously measured and recorded, and either the identity of the unknown can be determined or the sensor array can be trained to associate the measured response with a particular analyte which is then added to the library of response signatures.

The present invention overcomes certain limitations of the current art by embodying the innovation of a self-encoding sensor array wherein a characteristic optical response signature is produced by the interaction of specific bead subpopulation compositions with a reporter dye. In the self-encoding sensor array of the present invention, the response signal to a target analyte serves both as a response signature for the target analyte and as the encoding signal for the entire sensor array and subpopulations within the array. The decoding of the array is thus accomplished in a one-step process during the array response measurement of a target analyte and utilizes the very response which is used to identify the target analyte. The bead encoding is thus incorporated into the array by the nature of the bead subpopulation responses to target analytes.

In one preferred embodiment, the distal end of a fiber optic bundle substrate is chemically etched so as to create a cavity or micro-well at the end of a discrete fiber. In the preferred embodiment, each one of the beads is located within separate microwells formed at terminal ends of optical fibers of the bundle. These microwells are formed by anisotropic etching of the cores of the optical fibers with respect to the cladding. The resultant etched cavity is dimensioned for accommodating an individual microbead sensor and for providing optical coupling of the individual bead sensor with the discrete optical fiber in the fiber bundle. Since typical fiber optic bundles contain thousands of discrete fibers, this embodiment provides for the individual optical coupling of thousands of sensors in a sensor array, thereby providing for a large number of independent sensor measurements for each bead subpopulation within the array.

Due to both the large number of bead sensor subpopulations available and the correspondingly large number of sensor elements within each subpopulation, a significant innovation of the present invention is in providing for thousands of independent sensor response measurements in a single sensor array. This enables another significant innovation of the present invention by providing for the summing and amplification of the characteristic optical response signatures of multiple independent measurements taken from sensor beads within each sensor array bead subpopulation. This approach directly mimics the actual behavior of the human olfactory where the combined signals from thousands of receptor cells in each of grouping of nearly a thousand different receptor cell types found in the epithelium layer, none of which are particularly sensitive in themselves, lead to a highly amplified sensory response to odors [see J.S. Kauer, *Trends Neurosci.* 14:79-95(1991)].

The present invention thus embodies the evolutionary scent amplification process found in the human olfactory system in order to significantly enhance sensor array sensitivity to analytes by summing the low-level responses of a large number of sensor array elements. By summing the responses from several beads at low vapor concentrations, a substantial improvement in signal-to-noise ratios is achieved, exceeding a factor of ten or more. This innovation has led to reducing the detection limit of the sensor array by over an order of magnitude. The enhancement in sensitivity provided by the sensor array of the present invention is generally known to be directly proportional to the square root of the number of independent sensor bead responses available for summing. With such enhancements, detection limits approaching parts per billion are achievable.

In preferred embodiments, the sensor beads are self-encoded using a reporter dye that is preferably infiltrated or entrapped within the beads. The reporter dye may be a chromophore or phosphor but is preferably a fluorescent dye, which due to characteristically strong optical signals provide a good signal-to-noise ratio for decoding. Although not necessary, the self-encoding can also be accomplished by utilizing the ratios of two or more reporting dyes having characteristic and discrete emission peaks and measuring the peak intensity ratios upon illumination with excitation light energy.

some arrays, multiple substrates may be used, either of different or identical compositions. Thus for example, large arrays may comprise a plurality of smaller substrates.

In addition, one advantage of the present compositions is that particularly through the use of fiber optic technology, extremely high density arrays can be made. Thus for example, because beads of 200 nm can be used, and very small fibers are known, it is possible to have as many as 250,000 different fibers and beads in a 1 mm^2 fiber optic bundle, with densities of greater than 15,000,000 individual beads and fibers per 0.5 cm^2 obtainable.

The compositions comprise a substrate. By "substrate" or "solid support" or other grammatical equivalents herein is meant any material that can be modified to contain discrete individual sites appropriate for the attachment or association of beads and is amenable to at least one detection method. As will be appreciated by those in the art, the number of possible substrates are very large, and include, but are not limited to, glass and modified or functionalized glass, plastics (including acrylics, polystyrene and copolymers of styrene and other materials, polypropylene, polyethylene, polybutylene, polyurethanes, Teflon, etc.), polysaccharides, nylon or nitrocellulose, resins, silica or silica-based materials including silicon and modified silicon, carbon, metals, inorganic glasses, plastics, optical fiber bundles, and a variety of other polymers. In general, the substrates allow optical detection and do not appreciably fluoresce.

Generally the substrate is flat or planar, although as will be appreciated by those in the art, other configurations of substrates may be used as well; for example, three dimensional configurations can be used, for example by embedding the beads in a porous block of plastic that allows sample access to the beads and using a confocal microscope for detection. Similarly, the beads may be placed on the inside surface of a tube, for flow-through sample analysis to minimize sample volume. Preferred substrates include optical fiber bundles as discussed below, and flat planar substrates such as glass, polystyrene and other plastics and acrylics.

At least one surface of the substrate is modified to contain discrete, individual sites for later association of microspheres. These sites may comprise physically altered sites, i.e. physical configurations such as wells or small depressions in the substrate that can retain the beads, such that a microsphere can rest in the well, or the use of other forces (magnetic or compressive), or chemically altered or active sites, such as chemically functionalized sites, electrostatically altered sites, hydrophobically/ hydrophilically functionalized sites, spots of adhesive, etc.

The sites may be a pattern, i.e. a regular design or configuration, or randomly distributed. A preferred embodiment utilizes a regular pattern of sites such that the sites may be addressed in the X-Y coordinate plane. "Pattern" in this sense includes a repeating unit cell, preferably one that allows a high density of beads on the substrate. However, it should be noted that these sites may not be

hydrophobic sites with hydrophobic beads, in an aqueous system, drives the association of the beads preferentially onto the sites. As outlined above, "pattern" in this sense includes the use of a uniform treatment of the surface to allow attachment of the beads at discrete sites, as well as treatment of the surface resulting in discrete sites. As will be appreciated by those in the art, this may be accomplished in a variety of ways.

The compositions of the invention further comprise a population of microspheres. By "population" herein is meant a plurality of beads as outlined above for arrays. Within the population are separate subpopulations, which can be a single microsphere or multiple identical microspheres. That is, in some embodiments, as is more fully outlined below, the array may contain only a single bead for each bioactive agent; preferred embodiments utilize a plurality of beads of each type.

By "microspheres" or "beads" or "particles" or grammatical equivalents herein is meant small discrete particles. The composition of the beads will vary, depending on the class of bioactive agent and the method of synthesis. Suitable bead compositions include those used in peptide, nucleic acid and organic moiety synthesis, including, but not limited to, plastics, ceramics, glass, polystyrene, methylstyrene, acrylic polymers, paramagnetic materials, thoria sol, carbon, graphite, titanium dioxide, latex or cross-linked dextrans such as Sepharose, cellulose, nylon, cross-linked micelles and teflon may all be used.

Synthetic beads may be fabricated by polymerizing or copolymerizing a variety of condensation or vinyl precursor monomers or by way of combinatorial polymer synthesis. Such polymers can be further modified by the addition of plasticizers, such as tritolyl phosphate (TTP), triphenyl phosphate (TTP) or dibutyl phthalate (DBP). Particularly useful dye-encoding bead candidates for use in sensor array subpopulations are polymer and copolymer materials which exhibit either a characteristic swelling upon exposure to various vapor analytes, a characteristic polarity difference due to their chemical structure, or a characteristic chemical adsorption response with various vapor analytes. In prescreening candidate polymers as bead materials and evaluating candidates based on desirable swelling, polarity and adsorption characteristics, two particularly useful references are: R.A. McGill, et al., *Chemtech*, September 24, 1996,p27-37 and J.W. Grate, et al., *Anal. Chem.* 68:913-7 (1996).

A variety of bead chemistries may be utilized in fabricating a wide diversity of sensor bead subpopulations. For example, the following compositions have been found to be particularly useful as candidate bead materials: silica, poly(ethylene glycol), polycaprolactone, poly(1,4-butylene adipate), PDPO [poly(2,6-dimethyl-1,4-phenyleneoxide)], PS078.5 [triethoxysilyl-modified polybutadiene (50% in toluene)], PS078.8 [diethoxymethylsilyl-modified polybutadiene in toluene], CPS2067 [acryloxypropylmethyl-cyclosiloxane], PS802 [(80-85%) dimethyl-(15-20%) (acryloxypropyl) methylsiloxane copolymer], PS901.5 poly(acryloxypropyl-methyl)siloxane], PS851 [(97-98%) dimethyl-(2-3%) (methacryloxypropyl) methylsiloxane copolymer], PABS [poly(acrylonitrile-butadiene-styrene)],

microspheres (i.e. each sensor element) comprises a unique optical response signature or optical tag, that can be used to identify the unique bioactive agent or chemical functionality of that subpopulation of microspheres; a bead comprising the unique optical response signature may be distinguished from beads at other locations with different optical response signatures. As is outlined herein, each bioactive agent will have an associated unique optical response signature such that any microspheres comprising that bioactive agent will be identifiable on the basis of the signature upon exposure to a reference analyte or fluid. As is more fully outlined below, it is possible to reuse or duplicate optical response signatures within an array, for example, when another level of identification is used, for example when beads of different sizes are used, or when the array is loaded sequentially with different batches of beads.

The selection of chemical dye indicators is equally important to the design of a fiber optic sensor array system of the present invention. In the preferred embodiment (see Fig. 1), at least one dye 11 is incorporated into the microsphere 10. In the preferred embodiment, this dye 11 acts as both an encoding dye, for identifying the bead subpopulation location in the sensor array, and a reporting dye, for detecting a target analyte of interest. In an alternative embodiment, two or more dyes may be utilized as encoding-reporter dyes. In a preferred embodiment, at least one dye is used solely as an encoding dye and a separate dye is added during analysis as a reporting dye. In one embodiment, where two or more encoding dyes are used, the ratio of peak intensities for dye pairs may be used for encoding the bead subpopulation and a separate reporter dye may be added during analysis. In an alternative embodiment, conjugated dyes, such as acrlyoyl fluorescein and others, may be utilized where it is desirable to incorporate the dye directly into a synthesized polymer or copolymer bead material.

While the reporter dye 11 may be either a chromophore-type or a fluorophore-type, a fluorescent dye is preferred because the strength of the fluorescent signal provides a better signal-to-noise ratio when decoding. In the most preferred embodiment, polarity-sensitive dyes or solvatochromic dyes are utilized. Solvatochromic dyes are dyes whose absorption or emission spectra are sensitive to and altered by the polarity of their surrounding environment. Typically, these dyes exhibit a shift in peak emission wavelength due to a change in local polarity. Polarity changes which cause such wavelength shifts can be introduced by the bead matrix used for a particular sensor bead subpopulation or, the presence of a target analyte. The change in polarity creates a characteristic optical response signature which is useful for both encoding subpopulations of bead types and for detecting specific target analytes. One preferred solvatochromic dye, Nile Red (Eastman Kodak, Rochester, NY), exhibits large shifts in its emission wavelength peak with changes in the local environment polarity. In addition, Nile Red is soluble in a wide range of solvents, is photochemically stable, and has a relatively strong fluorescence peak. Additional dyes which are conventionally known in the art and may be used as dyes in the present invention are those found in U.S. Patent 5,512,490 to Walt, et al., of which Table 3, Table 4, Table 5, Table 6 and Table 11 are incorporated herein by reference.

50 are mixed in step 52 with dye solutions prepared in step 51. Preferably, in step 52, the beads or microspheres are placed in a dye solution comprising dye dissolved in an organic solvent that will swell the microspheres. In step 54, the beads are washed, centrifuged or filtered to remove excess dye. The microspheres are typically washed in water, methanol, or any suitable solvent that does not swell the microspheres, but in which the dyes are still soluble. This allows the residual dye to be rinsed off without rinsing the dye out of the microspheres. In an alternative embodiment, a chemical moiety or functional group may be attached to the bead surface following removal of excess dye.

The beads need not be spherical; irregular particles may be used. While both porous and non-porous beads may be utilized, porous beads are preferred for infiltrating the reporter dye and enhancing the responsivity and sensitivity of the microsphere sensor due to an increase in surface area for attachment of the reporter dye, bioactive agents, etc. The bead sizes range from nanometers, i.e. 100 nm, to millimeters, i.e. 1 mm, with beads from about 0.2 micron to about 200 microns being preferred, and from about 0.5 to about 5 micron being particularly preferred, although in some embodiments smaller beads may be used. Fig. 1 illustrates the construction of a typical bead or microsphere sensor 10 comprising a reporting dye 11 entrapped within bead pores 12.

It should be noted that a key component of the invention is the use of a substrate/bead pairing that allows the association or attachment of the beads at discrete sites on the surface of the substrate, such that the beads do not move during the course of the assay.

The present invention embodies a bead-based analytical chemistry system in which beads or microspheres are fabricated from various inorganic or organic materials wherein each material can be identified by a characteristic temporal optical response signature which enables verifying both the identity and location of a particular bead within a sensor array upon exposure to a reference analyte while illuminating with excitation light energy. The invention provides for utilization of any source of excitation light energy and is not limited to a specific wavelength. The principal requirement of the excitation light is that it produces emitted light of a characteristic wavelength upon illumination of a reporter dye associated with a given bead composition.

In a preferred embodiment, the microspheres further comprise a bioactive agent. By "candidate bioactive agent" or "bioactive agent" or "chemical functionality" or "binding ligand" herein is meant as used herein describes any molecule, *e.g.*, protein, oligopeptide, small organic molecule, polysaccharide, polynucleotide, etc. which can be attached to the microspheres of the invention. It should be understood that the compositions of the invention have two primary uses. In a preferred embodiment, as is more fully outlined below, the compositions are used to detect the presence of a particular target analyte; for example, the presence or absence of a particular nucleotide sequence or a particular protein, such as an enzyme, an antibody or an antigen. In an alternate preferred

preferred in this embodiment are libraries of bacterial, fungal, viral, and mammalian proteins, with the latter being preferred, and human proteins being especially preferred.

In a preferred embodiment, the bioactive agents are peptides of from about 5 to about 30 amino acids, with from about 5 to about 20 amino acids being preferred, and from about 7 to about 15 being particularly preferred. The peptides may be digests of naturally occurring proteins as is outlined above, random peptides, or "biased" random peptides. By "randomized" or grammatical equivalents herein is meant that each nucleic acid and peptide consists of essentially random nucleotides and amino acids, respectively. Since generally these random peptides (or nucleic acids, discussed below) are chemically synthesized, they may incorporate any nucleotide or amino acid at any position. The synthetic process can be designed to generate randomized proteins or nucleic acids, to allow the formation of all or most of the possible combinations over the length of the sequence, thus forming a library of randomized bioactive proteinaceous agents.

In a preferred embodiment, a library of bioactive agents are used. The library should provide a sufficiently structurally diverse population of bioactive agents to effect a probabilistically sufficient range of binding to target analytes. Accordingly, an interaction library must be large enough so that at least one of its members will have a structure that gives it affinity for the target analyte. Although it is difficult to gauge the required absolute size of an interaction library, nature provides a hint with the immune response: a diversity of 10^7-10^8 different antibodies provides at least one combination with sufficient affinity to interact with most potential antigens faced by an organism. Published in vitro selection techniques have also shown that a library size of 10^7 to 10^8 is sufficient to find structures with affinity for the target. Thus, in a preferred embodiment, at least 10^6, preferably at least 10^7, more preferably at least 10^8 and most preferably at least 10^9 different bioactive agents are simultaneously analyzed in the subject methods. Preferred methods maximize library size and diversity.

In one embodiment, the library is fully randomized, with no sequence preferences or constants at any position. In a preferred embodiment, the library is biased. That is, some positions within the sequence are either held constant, or are selected from a limited number of possibilities. For example, in a preferred embodiment, the nucleotides or amino acid residues are randomized within a defined class, for example, of hydrophobic amino acids, hydrophilic residues, sterically biased (either small or large) residues, towards the creation of cysteines, for cross-linking, prolines for SH-3 domains, serines, threonines, tyrosines or histidines for phosphorylation sites, etc., or to purines, etc.

In a preferred embodiment, the bioactive agents are nucleic acids (generally called "probe nucleic acids" or "candidate probes" herein). By "nucleic acid" or "oligonucleotide" or grammatical equivalents herein means at least two nucleotides covalently linked together. A nucleic acid of the present invention will generally contain phosphodiester bonds, although in some cases, as outlined below, nucleic acid analogs are included that may have alternate backbones, comprising, for example,

herein), such that hybridization of the target and the probes of the present invention occurs. This complementarity need not be perfect; there may be any number of base pair mismatches that will interfere with hybridization between the target sequence and the single stranded nucleic acids of the present invention. However, if the number of mutations is so great that no hybridization can occur under even the least stringent of hybridization conditions, the sequence is not a complementary target sequence. Thus, by "substantially complementary" herein is meant that the probes are sufficiently complementary to the target sequences to hybridize under the selected reaction conditions. High stringency conditions are known in the art; see for example Maniatis et al., Molecular Cloning: A Laboratory Manual, 2d Edition, 1989, and Short Protocols in Molecular Biology, ed. Ausubel, et al., both of which are hereby incorporated by reference. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g. 10 to 50 nucleotides) and at least about 60°C for long probes (e.g. greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. In another embodiment, less stringent hybridization conditions are used; for example, moderate or low stringency conditions may be used, as are known in the art; see Maniatis and Ausubel, supra, and Tijssen, supra.

The term 'target sequence" or grammatical equivalents herein means a nucleic acid sequence on a single strand of nucleic acid. The target sequence may be a portion of a gene, a regulatory sequence, genomic DNA, cDNA, RNA including mRNA and rRNA, or others. It may be any length, with the understanding that longer sequences are more specific. As will be appreciated by those in the art, the complementary target sequence may take many forms. For example, it may be contained within a larger nucleic acid sequence, i.e. all or part of a gene or mRNA, a restriction fragment of a plasmid or genomic DNA, among others. As is outlined more fully below, probes are made to hybridize to target sequences to determine the presence or absence of the target sequence in a sample. Generally speaking, this term will be understood by those skilled in the art.

In a preferred embodiment, the bioactive agents are organic chemical moieties, a wide variety of which are available in the literature.

Table I

Surface chemistry	Name:
NH_2	Amine
COOH	Carboxylic Acid
CHO	Aldehyde
CH_2-NH_2	Aliphalic Amine
CO NH_2	Amide
CH_2-C1	Chloromethyl
CONH-NH_2	Hydrazide
OH	Hydroxyl
SO_4	Sulfate
SO_3	Sulfonate
Ar NH_2	Aromatic Amine

et al., Critical Rev. Therapeutic Drug Carrier Systems, 7(4):275-308 (1991), expressly incorporated herein). Proteinaceous bioactive agents may also be attached using other techniques known in the art, for example for the attachment of antibodies to polymers; see Slinkin et al., Bioconj. Chem. 2:342-348 (1991); Torchilin et al., *supra*; Trubetskoy et al., Bioconj. Chem. 3:323-327 (1992); King et al., Cancer Res. 54:6176-6185 (1994); and Wilbur et al., Bioconjugate Chem. 5:220-235 (1994), all of which are hereby expressly incorporated by reference). It should be understood that the bioactive agents may be attached in a variety of ways, including those listed above. What is important is that manner of attachment does not significantly alter the functionality of the bioactive agent; that is, the bioactive agent should be attached in such a flexible manner as to allow its interaction with a target.

Specific techniques for immobilizing enzymes on microspheres are known in the prior art. In one case, NH_2 surface chemistry microspheres are used. Surface activation is achieved with a 2.5% glutaraldehyde in phosphate buffered saline (10 mM) providing a pH of 6.9. (138 mM NaCl, 2.7 mM, KCl). This is stirred on a stir bed for approximately 2 hours at room temperature. The microspheres are then rinsed with ultrapure water plus 0.01% tween 20 (surfactant) -0.02%, and rinsed again with a pH 7.7 PBS plus 0.01% tween 20. Finally, the enzyme is added to the solution, preferably after being prefiltered using a 0.45μm amicon micropure filter.

These two components act in concert to properly position the proximal end 214 of the bundle 202 for a microscope objective lens 220. Light collected by the objective lens 220 is passed to a reflected light fluorescence attachment with a four position dichromic cube wheel 222. The attachment 222 allows insertion of light from a 75 Watt Xenon arc lamp 224 through the objective lens 220 to be coupled into the fiber bundle 202. The light from the source 224 is condensed by condensing lens 226, then filtered and/or shuttered by filter and shutter wheel 228.

Light returning from the distal end 212 of the bundle 202 is passed by the attachment 222 and is then shuttered and filtered by a second wheel 234. The light is then imaged on a charge coupled device (CCD) camera 236. A conventional computer 238 executes imaging processing software to process the information from the CCD camera 236 and also possibly control the first and second shutter and filter wheels 228, 234. Either a Macintosh or, alternatively, an IBM-compatible personal computer may be utilized for controlling the instrumentation and data acquisition. The instrumentation and optical apparatus deployed at the proximal end 214 of the fiber optic sensor 200, exclusive of the fiber optic sensor 200, are discussed more completely by Bronk, et al., *Anal. Chem.* 67(17):2750-2752(1995) and Bronk, et al., *Anal. Chem.* 66:3519 (1994).

The bead sensor array 100 may be attached to the distal end of the optical fiber bundle 202 using a variety of compatible processes. It is important that the microspheres 10 are located close to the end of the bundle. This ensures that the light returning in each discrete optical fiber predominantly comes from only a single microsphere. This feature is necessary to decode the self-encoded bead subpopulations for the purpose of identifying both bead type and location, to enable the interrogation of the optical signature of individual microspheres within a bead subpopulation, and to provide for the summing of individual bead responses within each subpopulation for reducing signal to noise and improving signal enhancement. The bead adhesion or affixing technique, however, must not chemically insulate the microspheres from the analyte or interfere with the optical measurement.

Figs. 5A and 5B are micrographs illustrating the preferred method for attaching beads to a sensor array substrate. Microwells 250 are formed on the distal end 212 of a fiber optic bundle 202 and microspheres 10 are inserted in the microwell cavities 250. The microwells 250 are formed at the center of each optical fiber 252 of the fiber optic bundle 202. As shown in Fig. 5B, the size of the microwells 250 are coordinated with the size of the microspheres 10 so that the microspheres 10 can be placed within the microwells 250. Thus, each optical fiber 252 of the bundle 202 conveys light from the single microsphere 10 contained in its well. Consequently, by imaging the end of the bundle 202 onto the CCD array 236, the optical signatures of the microspheres 10 are individually interrogatable.

Fig. 4 illustrates how the microwells 250 are formed and microspheres 10 placed in the microwells. In one embodiment, a 1 mm hexagonally-packed imaging fiber bundle 202 was employed comprising approximately 20,600 individual optical fibers having cores approximately 3.7μm across

In an another embodiment, an alternative fixation approach employs microsphere swelling to entrap each microsphere 10 in its corresponding microwell 250. In this approach, the microspheres are first distributed into the microwells 250 by sonicating the microspheres suspended in a non-swelling solvent in the presence of the microwell array on the distal end 212. After placement into the microwells, the microspheres are subsequently exposed to an aqueous buffer in which they swell, thereby physically entrapping them in the microwells. By way of example of this particular embodiment, one commonly known microsphere material is tentagel, a styrene-polyethylene glycol copolymer. These microspheres can be unswollen in nonpolar solvents such as hexane and swell approximately 20-40% in volume upon exposure to a more polar or aqueous media. In certain embodiments, this fixation approach may be desirable since it does not significantly compromise the diffusional or permeability properties of the microspheres themselves.

Figs. 6A and 6B show typical microspheres 10 in microwells 250 after their initial placement and then after tapping and exposure to air pulses. Figs. 6A and 6B illustrate that there is no appreciable loss of microspheres from the microwells due to mechanical agitation even without a specific fixing technique. This effect is probably due to electrostatic forces between the microspheres and the optical fibers. These forces tend to bind the microspheres within the microwells. Thus, in most environments, it may be unnecessary to use any chemical or mechanical fixation for the microspheres.

It should be noted that not all sites of an array may comprise a bead; that is, there may be some sites on the substrate surface which are empty. In addition, there may be some sites that contain more than one bead, although this is not preferred.

In some embodiments, for example when chemical attachment is done, it is possible to attach the beads in a non-random or ordered way. For example, using photoactivatible attachment linkers or photoactivatible adhesives or masks, selected sites on the array may be sequentially rendered suitable for attachment, such that defined populations of beads are laid down.

In addition, since the size of the array will be set by the number of unique optical response signatures, it is possible to "reuse" a set of unique optical response signatures to allow for a greater number of test sites. This may be done in several ways; for example, by using a positional coding scheme within an array; different sub-bundles may reuse the set of optical response signatures. Similarly, one embodiment utilizes bead size as a coding modality, thus allowing the reuse of the set of unique optical response signatures for each bead size. Alternatively, sequential partial loading of arrays with beads can also allow the reuse of optical response signatures.

In a preferred embodiment, a spatial or positional coding system is done. In this embodiment, there are sub-bundles or subarrays (i.e. portions of the total array) that are utilized. By analogy with the telephone system, each subarray is an "area code", that can have the same tags (i.e. telephone

immuno-based sensors (both of which are part of a broader general class of protein sensors); and 4) geno-sensors.

In a preferred embodiment, the bioactive agents are used to detect chemical compounds. A large number of basic indicator sensors have been previously demonstrated. Examples include:

Table III

TARGET ANALYTE	Bioactive agent (or chemical functionality)	NOTES ($\lambda_{AB}/\lambda_{EM}$)
pH Sensors based on:	seminaphthofluoresceins	*e.g.*, carboxyl-SNAFL
	seminaphthorhodafluors	*e.g.*, carboxyl-SNARF
	8-hydroxypyrene-1,3,6-trisulfonic acid	
	fluorescein	
CO2 Sensors based On:	seminaphthofluoresceins	*e.g.*, carboxyl-SNAFL
	seminaphthorhodafluors	*e.g.*, carbody-SNARF
	8-hydroxypyrene-1,3,6-trisulfonic acid	
Metal Ions Sensors based on:	desferriozamine B	*e.g.*, Fe
	cyclen derivative	*e.g.*, Cu, Zn
	derivatized peptides	*e.g.*, FITC-Gly-Gly-His, and FITC-Gly His, Cu, Zn
	fluorexon (calcine)	*e.g.*, Ca, Mg, Cu, Pb, Ba
	calcine blue	*e.g.*, Ca, Mg, Cu
	methyl calcine blue	*e.g.*, Ca, Mg, Cu
	ortho-dianisidine tetracetic acid (ODTA)	*e.g.*, Zn
	bis-salicylidene ethylenediamine (SED)	*e.g.*, Al

Table III		
Na^+	SBFI	339/565
	SBFO	354/575
	Sodium Green	506/535
K^+	PBFI	336/557
Cl^-	SPQ	344/443
	MQAE	350/460

Each of the chemicals listed in Table III directly produces an optically interrogatable signal or a change in the optical signature, as is more fully outlined below, in the presence of the targeted analyte.

Enzyme-based microsphere sensors have also been demonstrated and could be manifest on microspheres. Examples include:

Table IV

SENSOR TARGET	Bioactive agent
Glucose Sensor	glucose oxidase (enz.) + O_2-sensitive dye (see Table I)
Penicillin Sensor	penicillinase (enz.) + pH-sensitive dye (see Table I)
Urea Sensor	urease (enz.) + pH-sensitive dye (see Table I)
Acetylcholine Sensor	acetylcholinesterase (enz.) + pH-sensitive dye (see Table I)

Generally, as more fully outlined above, the induced change in the optical signal upon binding of the target analyte due to the presence of the enzyme-sensitive chemical analyte occurs indirectly in this class of chemical functionalities. The microsphere-bound enzyme, *e.g.*, glucose oxidase, decomposes the target analyte, *e.g.*, glucose, consume a co-substrate, *e.g.*, oxygen, or produce some by-product, *e.g.*, hydrogen peroxide. An oxygen sensitive dye is then used to trigger the signal change.

Immuno-based microsphere sensors have been demonstrated for the detection for environmental pollutants such as pesticides, herbicides, PCB's and PAH's. Additionally, these sensors have also

Table V

PROBE SEQUENCES	TARGET SEQUENCES
B-glo(+) (segment of human B-globin)5'-NH_2-$(CH_2)_8$-)TT TTT TTT TCA ACT TCA TCC ACG TTC ACC-3	B-glo(+)-CF 5'-Fluorescein-TC AAC GTG GAT GAA GTT C-3'
IFNG(interferon gamma 1)5'-NH_2-$(CH_2)_8$-T_{12}-TGG CTT CTC TTG GCT GTT ACT-3'	IFNG-CF 5'-Fluorescein-AG TAA CAG CCA AGA GAA CCC AAA-3'
IL2(interleukin-2)5'-NH_2-$(CH_2)_8$-T_{12}-TA ACC GAA TCC CAA ACT CAC CAG-3'	IL2-CF 5'-Fluorescein-CT GGT GAG TTT GGG ATT CTT GTA-3'
IL4(interleukin-4)5'NH_2-$(CH_2)_8$-T_{12}-CC AAC TGC TTC CCC CTC TGT-3'	IL4-CF 5'-Fluorescein-AC AGA GGG GGA AGC AGT TGG-3'
IL6(interleukin-6)5'NH_2-$(CH_2)_8$-T12-GT TGG GTC AGG GGT GGT TAT T-3'	IL6-CF 5'-Fluorescein-AA TAA CCA CCC CTG ACC CAA C-3'

In a preferred embodiment, the beads are loaded onto the substrate and then the array is decoded, prior to running the assay. This is done by detecting the optical response signature associated with the bead at each site on the array upon exposure to a reference analyte. This may be done all at once, if unique optical signatures are used, or sequentially, as is generally outlined above for the "reuse" of sets of optical signatures. Alternatively, full or partial decoding may occur after the assay is run.

Once made and decoded if necessary, the compositions find use in a number of applications. As a preliminary matter, the invention finds use in methods for reducing the signal-to-noise ratio in the characteristic optical response signature of a sensor array having a subpopulations of array elements. The methods comprise a) decoding the array so as to identify the location of each sensor element within each sensor subpopulation within the array; b) measuring the characteristic optical response signature of each sensor element in the array; c) adjusting the baseline of the optical response signature for each sensor element in the array; d) summing the baseline-adjusted characteristic optical response signature of all sensor elements within each of the sensor subpopulations; and e) reporting the characteristic optical response signature of each sensor subpopulation as a summation of the baseline-adjusted characteristic optical response signatures of all sensor elements within each of the

Furthermore, in some embodiments, a change in the optical response signature may be the basis of the optical signal. For example, the interaction of some chemical target analytes with some fluorescent dyes on the beads may alter the optical response signature, thus generating a different optical signal. For example, fluorophore derivatized receptors may be used in which the binding of the ligand alters the signal.

As will be appreciated by those in the art, in some embodiments, the presence or absence of the target analyte may be done using changes in other optical or non-optical signals, including, but not limited to, surface enhanced Raman spectroscopy, surface plasmon resonance, radioactivity, etc.

The assays may be run under a variety of experimental conditions, as will be appreciated by those in the art. A variety of other reagents may be included in the screening assays. These include reagents like salts, neutral proteins, e.g. albumin, detergents, etc which may be used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Also reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., may be used. The mixture of components may be added in any order that provides for the requisite binding. Various blocking and washing steps may be utilized as is known in the art.

In a preferred embodiment, the compositions are used to probe a sample solution for the presence or absence of a target analyte. By "target analyte" or "analyte" or grammatical equivalents herein is meant any atom, molecule, ion, molecular ion, compound or particle to be either detected or evaluated for binding partners. As will be appreciated by those in the art, a large number of analytes may be used in the present invention; basically, any target analyte can be used which binds a bioactive agent or for which a binding partner (i.e. drug candidate) is sought.

Suitable analytes include organic and inorganic molecules, including biomolecules. When detection of a target analyte is done, suitable target analytes include, but are not limited to, an environmental pollutant (including pesticides, insecticides, toxins, etc.); a chemical (including solvents, polymers, organic materials, etc.); therapeutic molecules (including therapeutic and abused drugs, antibiotics, etc.); biomolecules (including hormones, cytokines, proteins, nucleic acids, lipids, carbohydrates, cellular membrane antigens and receptors (neural, hormonal, nutrient, and cell surface receptors) or their ligands, etc); whole cells (including prokaryotic (such as pathogenic bacteria) and eukaryotic cells, including mammalian tumor cells); viruses (including retroviruses, herpesviruses, adenoviruses, lentiviruses, etc.); and spores; etc. Particularly preferred analytes are nucleic acids and proteins.

In a preferred embodiment, the target analyte is a protein. As will be appreciated by those in the art, there are a large number of possible proteinaceous target analytes that may be detected or evaluated for binding partners using the present invention. Suitable protein target analytes include, but are not

the use of single nucleotide polymorphisms (SNPs), which occur at an average frequency of more than 1 per kilobase in human genomic DNA. Some SNPs, particularly those in and around coding sequences, are likely to be the direct cause of therapeutically relevant phenotypic variants. There are a number of well known polymorphisms that cause clinically important phenotypes; for example, the apoE2/3/4 variants are associated with different relative risk of Alzheimer's and other diseases (see Cordor et al., Science 261(1993). Multiplex PCR amplification of SNP loci with subsequent hybridization to oligonucleotide arrays has been shown to be an accurate and reliable method of simultaneously genotyping at least hundreds of SNPs; see Wang et al., Science, 280:1077 (1998); see also Schafer et al., Nature Biotechnology 16:33-39 (1998). The compositions of the present invention may easily be substituted for the arrays of the prior art.

In a preferred embodiment, the compositions of the invention are used to screen bioactive agents to find an agent that will bind, and preferably modify the function of, a target molecule. As above, a wide variety of different assay formats may be run, as will be appreciated by those in the art. Generally, the target analyte for which a binding partner is desired is labeled; binding of the target analyte by the bioactive agent results in the recruitment of the label to the bead, with subsequent detection.

In a preferred embodiment, the binding of the bioactive agent and the target analyte is specific; that is, the bioactive agent specifically binds to the target analyte. By "specifically bind" herein is meant that the agent binds the analyte, with specificity sufficient to differentiate between the analyte and other components or contaminants of the test sample. However, as will be appreciated by those in the art, it will be possible to detect analytes using binding which is not highly specific; for example, the systems may use different binding ligands, for example an array of different ligands, and detection of any particular analyte is via its "signature" of binding to a panel of binding ligands, similar to the manner in which "electronic noses" work. This finds particular utility in the detection of chemical analytes. The binding should be sufficient to remain bound under the conditions of the assay, including wash steps to remove non-specific binding, although in some embodiments, wash steps are not desired; i.e. for detecting low affinity binding partners. In some embodiments, for example in the detection of certain biomolecules, the dissociation constants of the analyte to the binding ligand will be less than about 10^{-4}-10^{-6} M^{-1}, with less than about 10^{-5} to 10^{-9} M^{-1} being preferred and less than about 10^{-7} -10^{-9} M^{-1} being particularly preferred.

The following examples serve to more fully describe the manner of using the above-described invention, as well as to set forth the best modes contemplated for carrying out various aspects of the invention. It is understood that these examples in no way serve to limit the true scope of this invention, but rather are presented for illustrative purposes. All references cited herein are incorporated by reference in their entirety.

Fig.14	155	60
Fig.15	155	60
Fig.16	124	60

A conventional air dilution olfactometer and vacuum-controlled vapor delivery system 500, as commonly known and used in olfactory research and described in Kauer, et al., *J. Physiol.* 272:495-516 (1977), was used to apply controlled pulses of analyte vapor and air carrier gas to either a sensor bead substrate or the distal end 212 of a fiber optic sensor array 100 containing an array of sensor beads 10 immobilized in microwells 250.

To produce a saturated vapor sample, generally, a stream of air carrier gas is passed through a 5 ml cartridge containing filter paper saturated with the analyte. Analyte dilutions are produced by adjusting the relative flow rates of saturated vapor and clean carrier gas streams. Typically, a flow rate of 100 ml/min is used for the combined gas flow to the sensor array. At this flow rate, a 2 second pulse would deliver approximately 3.3 ml of analyte vapor with carrier gas. In generally, depending on the analyte vapor pressure and dilution factor, vapor pulses contain between 10^{-7} to 10^{-5} mol of analyte.

The vapor pulse was typically delivered during the 11th through 30th frame, commencing on the 11th frame. The duration of the vapor pulse varied with the specific frame rate utilized and typically ranged between 2 to 3 seconds. Baseline control measurements were performed with high purity, Ultra Zero grade air. The air pulse measurements were performed to account for any bead responses due to the vapor carrier gas.

Data processing: Following the collection of a temporal series of sensor bead or sensor array images, segments are drawn, using IPLab image processing software (Signal Analytics, Vienna, VA), over each pixel which corresponds to an individual fiber where the fiber is coupled to one sensor bead at its distal end. The mean fluorescence intensity was measured for each one of these segments in each frame in the sequence. This is done for both the vapor pulse responses and the baseline air pulse responses. Averages of multiple runs of each may be performed to improve data quality where needed. The air pulse data is then subtracted from the vapor pulse data to subtract the background due to air alone. The resulting data can be plotted to yield temporal intensity responses for all beads of interest. In a preferred embodiment, the sensor array data are used in a neural network analysis according to the method disclosed in White, et al, *Anal. Chem.* 68:2193-2202 (1996).

All data manipulation is performed within the IPLab program environment using simple operator scripts that call standardized image or data processing routines included with the software. These scripts and routines consist of a data collection portion and a data analysis portion.

In the data collection portion, there are three segments or loops as follows:

applying this baseline adjustment, when multiple bead responses are added together they can be amplified while the baseline remains at zero. Since all beads respond at the same time to the vapor pulse, they all see the pulse at the exact same time and there is no registering or adjusting needed for overlaying their responses. Cumulative response data is generated by simply adding all data points in successive time intervals. This final column, comprised of the sum of all data points at a particular time interval, may then be compared or plotted with the individual bead responses to determine the extent of signal enhancement or improved signal-to-noise ratios as shown in Figs. 14 and 15.

Example 1

Preparation of porous silica/Nile Red beads: Approximately 0.5 cm^3 of nominally 3.2 micron diameter commercial porous silica beads were removed from a LUNA column (Phenomenex, Torrance, CA). Sample of beads were placed onto a filter paper and, using vacuum filtration, 0.5 mL of Nile Red (Eastman Kodak, Rochester, NY) solution (1 mg/mL in toluene) was poured over beads. Nile Red was immediately taken up by silica beads, turning them a deep purple color. The beads were washed repeatedly with toluene to remove any excess, non-adsorbed Nile Red.. The beads were dried on a watch glass overnight. Beads were then placed into microwells formed by etching a fiber optic bundle according to the method of the present invention.

Example 2

Preparation of PDPO polymer coated porous silica beads: A silanizing solution was prepared from 20 uL N-octadecyl-triethyoxysilane in 980 uL of ethanol/water (95% ethanol, 5% ultrapure water with pH adjusted to 4.9 with acetic acid). The LUNA porous silica beads of Example 1 were dispersed in an excess of silanizing solution for approximately 10 minutes, vortexing continuously. The particles were rinsed three times with ethanol and dried in a 120°C oven, overnight for approximately 12 hours.

Stock solution of PDPO, poly(2,6-dimethyl-1,4-phenylene oxide),(Aldrich, Milwaukee, WI) and Nile Red was prepared from 0.09 g PDPO and 1.0 mL chloroform. After complete dissolution of the polymer, a 100 uL aliquot of 1mg/mL Nile red in chloroform was added. The resultant solution was vortexed continuously for uniform dispersion.

Excess PDPO/Nile Red was added to a small fraction of the silanized porous beads, approximately 100 uL polymer/dye solution to approximately 1mg of beads. The sample was vortexed for approximately 3 hours then washed. Excess polymer dye was removed and the beads were then washed repeatedly with methylene chloride, two to three times, followed by a washing with 0.01% polyoxyethylene-sorbitan monolaurate, Tween 20 (J.T.Baker, Cleveland, OH), in water. The washed beads were collected in a solution of 0.01% Tween 20/ultrapure water. A single, small drop was placed on a microscope coverslip and allowed to dry protected from light.

Example 5

Nile red dyed poly(methylstyrene/divinyl benzene) beads: Approximately 1 mg of commercially available 3.15 um polymer beads, 87% methyl styrene, 13% divinyl benzene with amine functionalized surface (Bangs Laboratories, Fishers, IN), was washed in 1 ml of methanol by vortexing, centrifuging at approximately 3000 rpm and decanting the solvent. The beads were transferred to brown vial and approximately 100 uL of Nile Red solution (1 mg/ml in toluene) was added. The sample was vortexed and placed on a wrist shaker to agitate overnight. The suspension was transferred to a microcentrifuge tube and washed with methanol until the decanted solvent was clear. The beads were collected in approximately 0.5 mL of a solution of 0.01% Tween 20 in ultrapure water. A single drop placed on a microscope coverslip and allowed to dry protected from light.

Example 6

Plasticizer modified poly(methylstyrene/divinyl benzene) beads with Nile red incorporated:

Approximately 1 mg of commercially available 3.15 um polymer beads, 87% methyl styrene, 13% divinyl benzene with amine functionalized surface (Bangs Laboratories, Fishers, IN), were rinsed with methanol according to Example 5 and transferred to a brown vial. Approximately 2-40% by wt plasticizer to polymer solutions of plasticizers, tritolyl phosphate (TTP), triphenyl phosphate (TPP),and dibutyl phthalate (DBP) (Aldrich, Milwaukee, WI), with Nile red solution (1 mg/mL in toluene) were added to samples of beads, covered, vortexed then shaken on wrist shaker for approximately 12 hours. The beads were transferred to microcentrifuge tubes and washed with Nile Red in methanol, then repeatedly with methanol until the decanted solvent was clear. The beads were collected in a solution of 0.01% Tween 20 in ultrapure water. A single drop of the suspension was placed on a microscope coverslip and allowed to dry protected from light.

Example 7

The porous silica beads prepared by the method of Example 1 were evaluated to determine their characteristic optical response signature to toluene vapor following the experimental method described above. The results are presented in Fig. 9 where the temporal optical response of 62 individual bead sensors to a pulse of toluene vapor is shown.

Example 8

The poly(methylstyrene/divinyl benzene) beads prepared by the method of Example 5 were evaluated to determine their characteristic optical response signature to methanol vapor. The results are

Example 13

The signal summing method of the present invention was evaluated in analyzing the experimental measurements made on poly(methylstyrene /divinyl benzene) beads prepared by the method of Example 5 and tested by the method of Example 8. The results are shown in Fig. 15 where the actual relative intensities of the temporal optical response for each of the 39 sensor beads is compared to relative intensity of the temporal optical response obtained from signal summing. As shown by Fig. 15, substantial signal enhancement is obtained by signal summing with a correspondingly significant improvement, up to a hundred fold, in the detection limit for target analytes.

Example 14

The polysiloxane coated porous silica beads prepared by the method of Example 3 were evaluated to determine their characteristic optical response signature to both toluene and methanol vapor. The results are presented in Fig. 16 where the temporal optical responses of two bead sensors to both toluene and methanol are shown. The results shown in Fig. 16 demonstrates the capability of this subpopulation of bead sensors to distinguish between two analytes of interest by utilizing the characteristic optical response signatures of the bead sensors to specific analytes.

Example 15

A 50/50 mixture of porous silica beads prepared by the method of Example 1 and poly(methylstyrene / divinyl benzene) beads prepared by the method of Example 5 were randomly dispersed and incorporated into etched microwells on the distal end of a fiber optic bundle according to the method of the present invention as described above. The resultant sensor array was evaluated to determine the characteristic optical response signature of the bead subpopulation to methanol vapor. An 535 nm excitation filter and 600 nm emission filter was used in this experiment. The results are presented in Fig. 17 where the normalized temporal optical response of 3 porous silica bead sensors and 6 PMS bead sensors to a pulse of methanol vapor is shown. In this example, the characteristic emitted light peak shapes of the bead subpopulations provide a distinguishable characteristic response signature for each subpopulation. Fig. 17 demonstrates the innovative self-encoding feature of the present invention where the identity and location of the beads is determined in a single measurement of a reference vapor analyte.

Example 16

The self-encoded fiber optic sensor array produced by the method of Example 15 was evaluated by measuring the characteristic temporal optical response signature of the porous silica and PMS sensor bead subpopulations of the array in response to a pulse of n-propanol vapor. The results are presented in Fig. 18 where the temporal optical response of 3 porous silica bead sensors and 6 PMS

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

ii) a population of microspheres comprising at least a first and a second subpopulation, each subpopulation comprising:

1) a bioactive agent; and

2) at least one reporter dye;

wherein said reporting dye has a first characteristic optical response signature when subjected to excitation light energy in the presence of a reference analyte;

wherein said microspheres are distributed on said surface;

b) detecting the presence of said analyte.

12. A method according to claim 11 further comprising identifying the location of each bioactive agent on said substrate by adding said reference analyte.

13. A method according to claim 11 or 12 wherein said detecting is done by detecting the presence of a label attached to said target analyte.

14. A method of decoding an array composition comprising

a) providing an array composition comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises at least one reporter dye; wherein said reporting dye has a first characteristic optical response signature when subjected to excitation light energy in the presence of a reference analyte; wherein said microspheres are distributed on said surface; and

b) adding at least one reference analyte to said array composition to identify the location of at least one subpopulation.

15. A method according to claim 14 wherein the location of each subpopulation is determined.

16. A self-encoding analytic chemical sensor array comprising:

a population of beads dispersed on a substrate, said population encoded with at least one reporting dye;

a plurality of separate subpopulations of beads contained within said population, each subpopulation of beads comprising a characteristic bead matrix material infiltrated with at least one reporting dye, which said reporting dye, in combination with said characteristic bead matrix material, has a first characteristic optical response signature when subjected to excitation light energy in the presence of a reference analyte and, which said reporting dye, in combination with

sites for hybridization.

25. The sensor array described in claim 16, wherein the substrate comprises a distal end of an optical fiber bundle.

26. The sensor array of claim 16, wherein the substrate comprises etched microwells at a distal end of individual fibers in an optical fiber bundle.

27. The sensor array of claim 26, wherein the etched wells form a cavity which is larger than the bead diameter and the bead is essentially disposed within said cavity.

28. The sensor array of claim 27 wherein said cavity is no greater than 5 um in diameter.

29. The sensor array of claim 16 wherein the average bead diameters are less than 4 um.

30. The sensor array of claim 16 wherein the characteristic bead matrix material is an organic material selected from the group consisting of polymers and copolymers.

31. The sensor array of claim 30 wherein the organic material swells upon contact to a target analyte.

32. The sensor array of claim 16 wherein the characteristic bead matrix material is an inorganic material selected from the group consisting of porous silicas, aluminas and zeolites.

33. A method for reducing the signal-to-noise ratio in the characteristic optical response signature of a sensor array having a subpopulations of array elements, comprising:

decoding the array so as to identify the location of each sensor element within each sensor subpopulation within the array;

measuring the characteristic optical response signature of each sensor element in the array;

adjusting the baseline of said optical response signature for each sensor element in said

a sensor array which comprises, a population of beads dispersed on a substrate, said population encoded with at least one reporting dye, a plurality of separate subpopulations of beads contained within said population, each subpopulation of beads comprising a characteristic bead matrix material infiltrated with at least one reporting dye, which said reporting dye, in combination with said characteristic bead matrix material, has a first characteristic optical response signature when subjected to excitation light energy in the presence of a reference analyte and, which said reporting dye, in combination with said characteristic bead matrix material, has a second characteristic optical response signature when subjected to excitation light energy in the presence of an unknown target analyte, wherein both the identity and the location of each bead comprising said bead subpopulations in said sensor array is determined and recorded upon exposure of said sensor array to said reference analyte while subjecting said bead to excitation light energy;

an optical fiber bundle, comprising a plurality of discrete individual fibers, each of said fibers in said bundle optically cooperating with and in optical communication with a discrete sensor bead in said sensor array;

an excitation light energy source in optical communication with a proximal end of said optical fiber bundle, said light source providing excitation light energy to said proximal end and said fiber bundle conveying said excitation light energy through said discrete fibers to a plurality of discrete sensor beads, wherein the light conveyed by each fiber is coupled to a specific bead element in said sensor array; and

an emission light energy detection means in optical communication with said proximal end of said fiber bundle, said detection means measuring light emitted from each fiber in said bundle at said proximal end in response to an analyte, wherein the emitted light which is conveyed by each fiber is produced from the response of a discrete sensor bead coupled to said fiber at a distal end of said fiber.

40. The sensing apparatus of claim 39 further comprising a neural network system for associating the emitted light response of said discrete sensor beads and a characteristic image of the emitted light response of the entire sensor array to a target analyte for the

produced from the response of a discrete sensor bead coupled to said fiber at a distal end of said fiber.

42. The sensing method of claim 41 further comprising a applying a neural network system for associating the emitted light response of said discrete sensor beads and a characteristic image of the emitted light response of the entire sensor array to a target analyte for the detection and identification of said analyte.

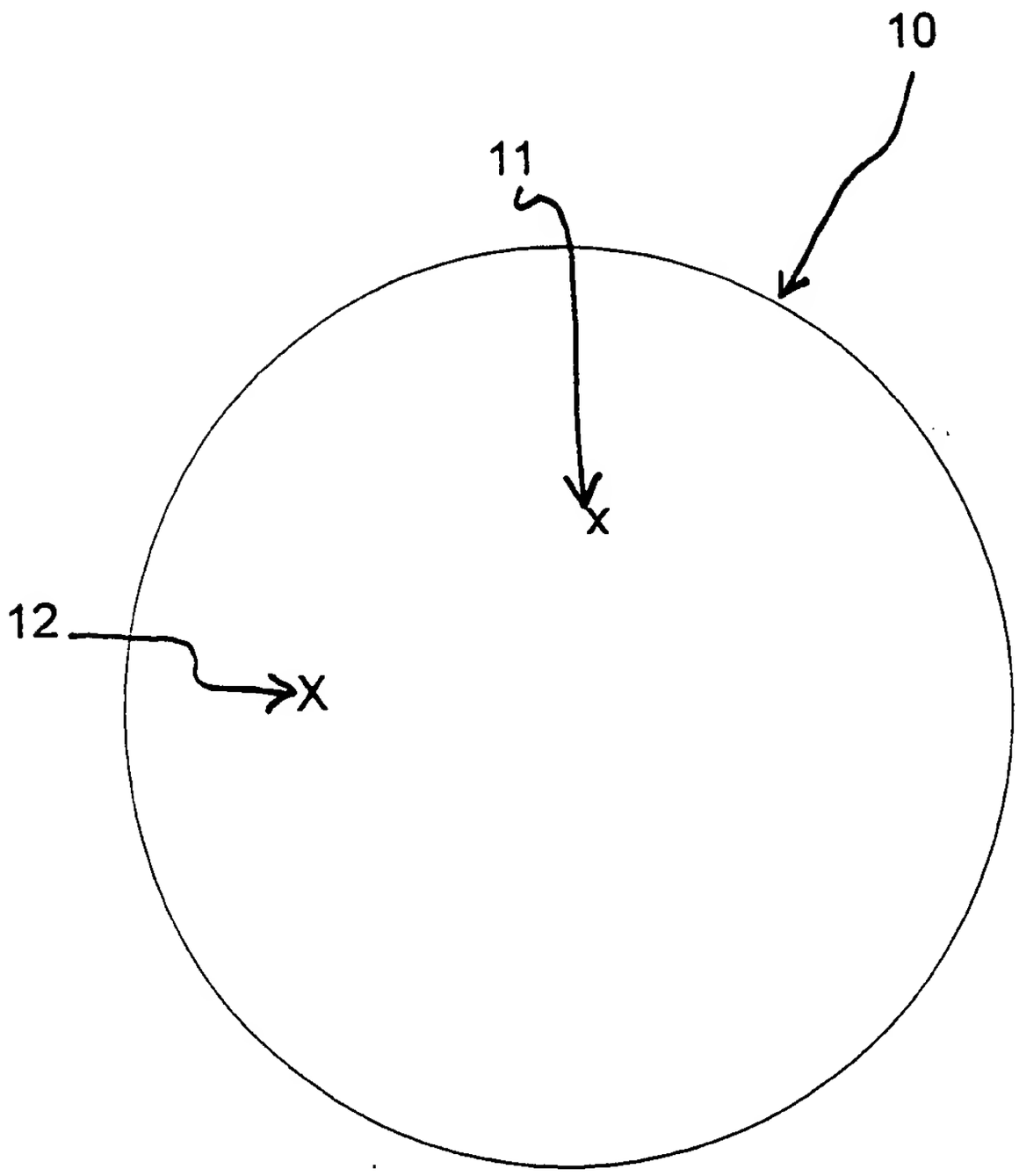


Fig. 1

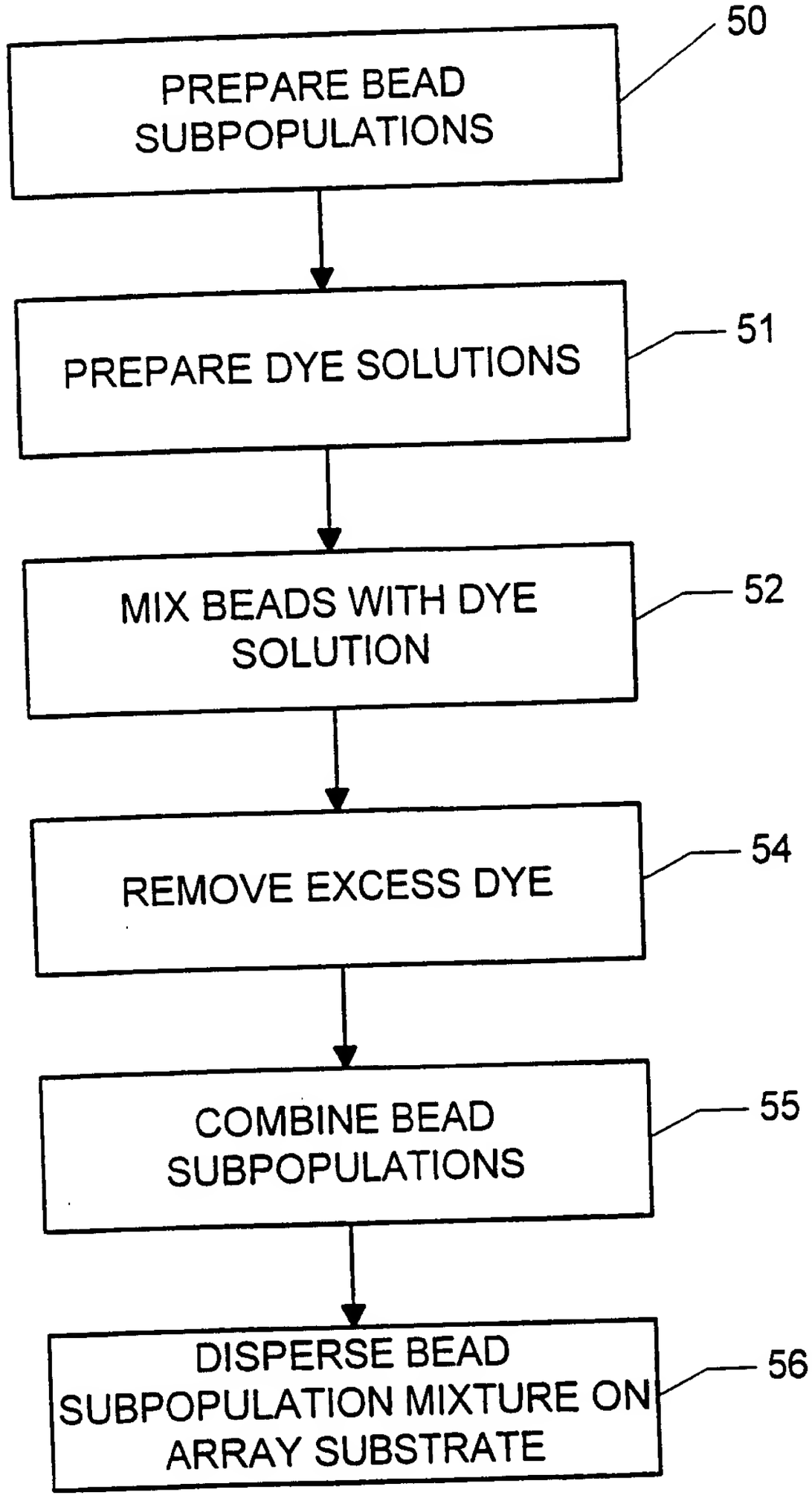

PREPARE BEAD SUBPOPULATIONS
50
PREPARE DYE SOLUTIONS
51
MIX BEADS WITH DYE SOLUTION
52
REMOVE EXCESS DYE
54
COMBINE BEAD SUBPOPULATIONS
55
DISPERSE BEAD SUBPOPULATION MIXTURE ON ARRAY SUBSTRATE
56

Fig. 2

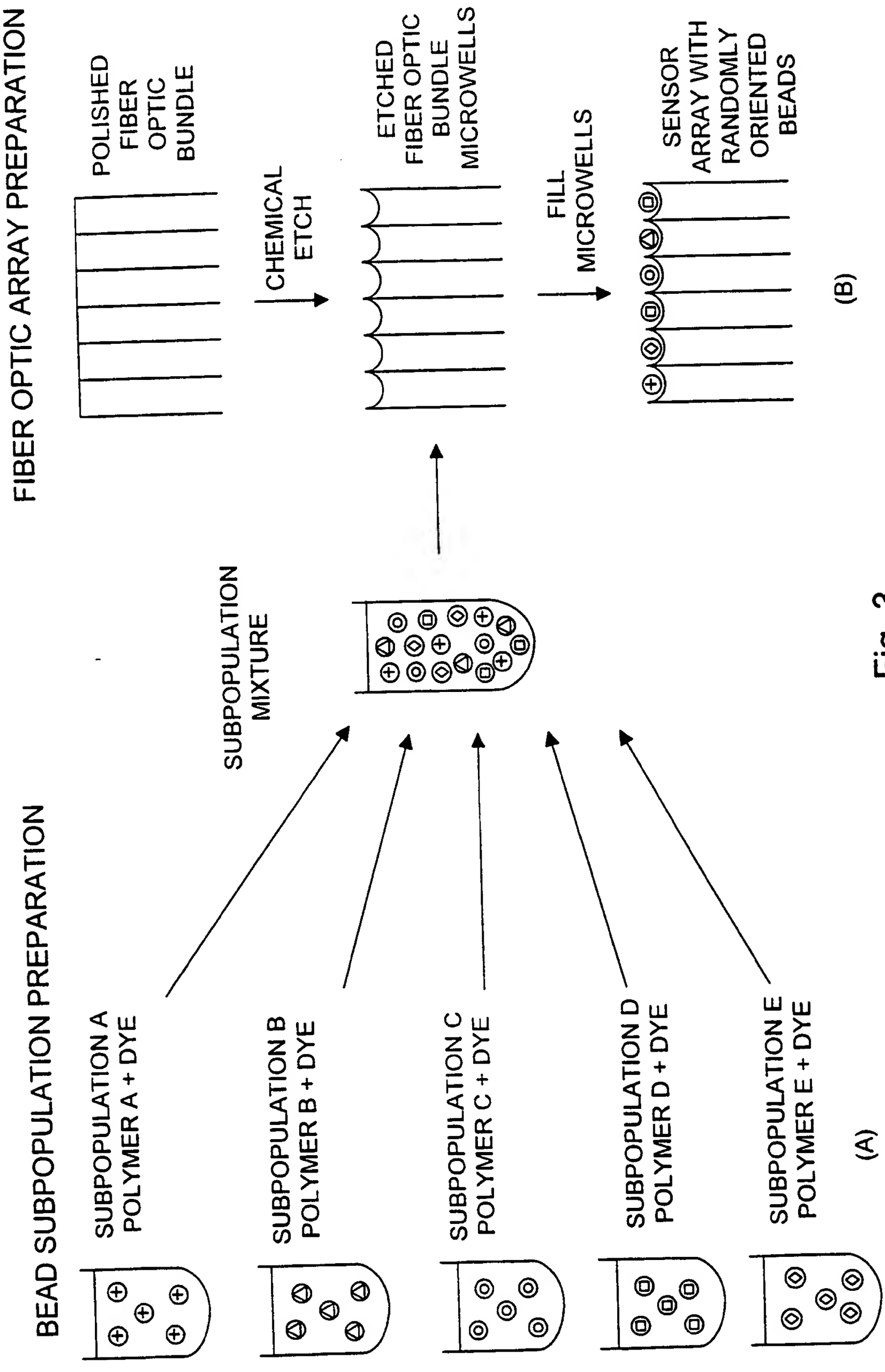

FIBER OPTIC ARRAY PREPARATION
POLISHED FIBER OPTIC BUNDLE
CHEMICAL ETCH
ETCHED FIBER OPTIC BUNDLE MICROWELLS
FILL MICROWELLS
SENSOR ARRAY WITH RANDOMLY ORIENTED BEADS
(B)
SUBPOPULATION MIXTURE
BEAD SUBPOPULATION PREPARATION
SUBPOPULATION A POLYMER A + DYE
SUBPOPULATION B POLYMER B + DYE
SUBPOPULATION C POLYMER C + DYE
SUBPOPULATION D POLYMER D + DYE
SUBPOPULATION E POLYMER E + DYE
(A)

Fig. 3

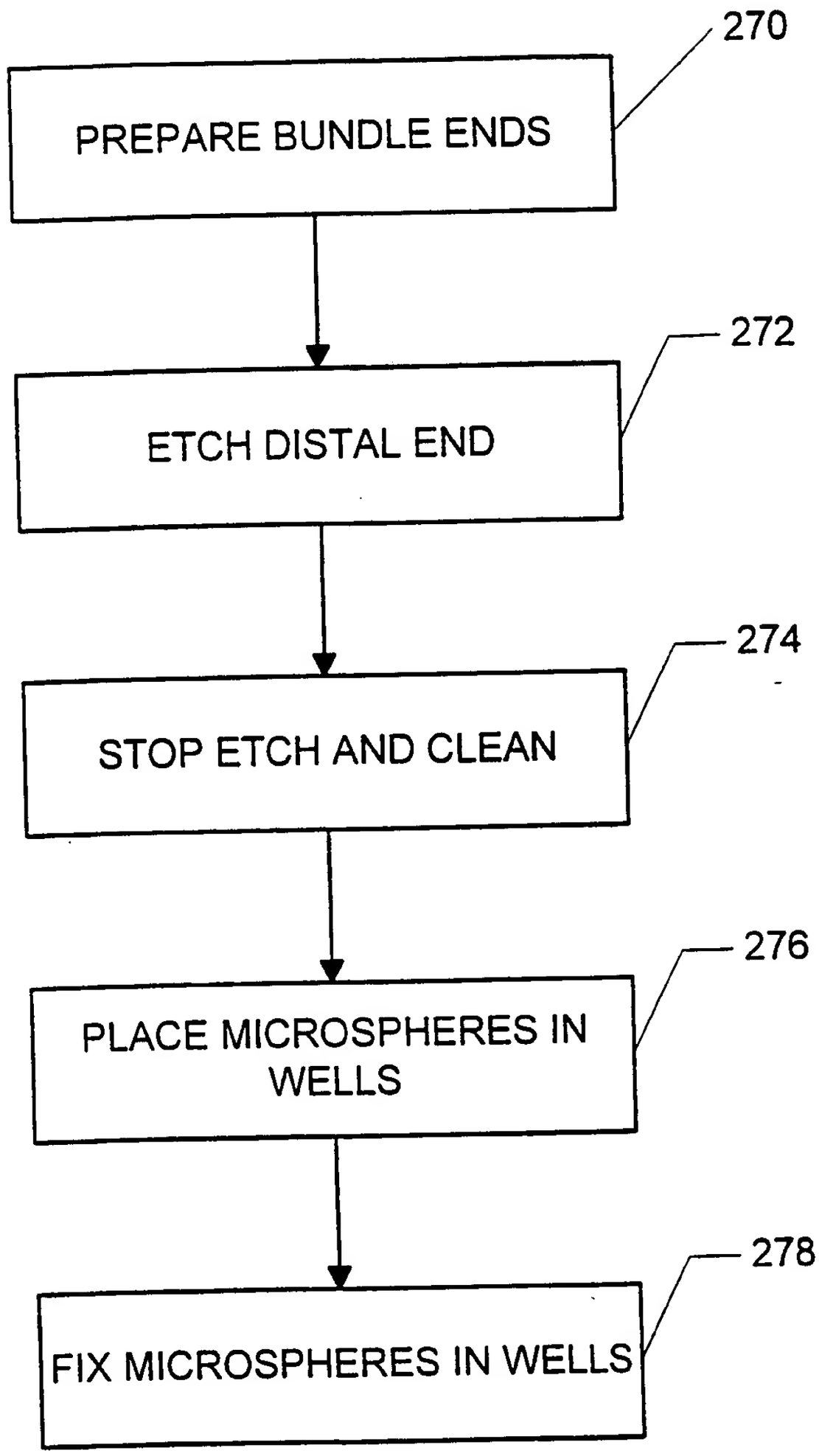

PREPARE BUNDLE ENDS
270
ETCH DISTAL END
272
STOP ETCH AND CLEAN
274
PLACE MICROSPHERES IN WELLS
276
FIX MICROSPHERES IN WELLS
278

Fig. 4

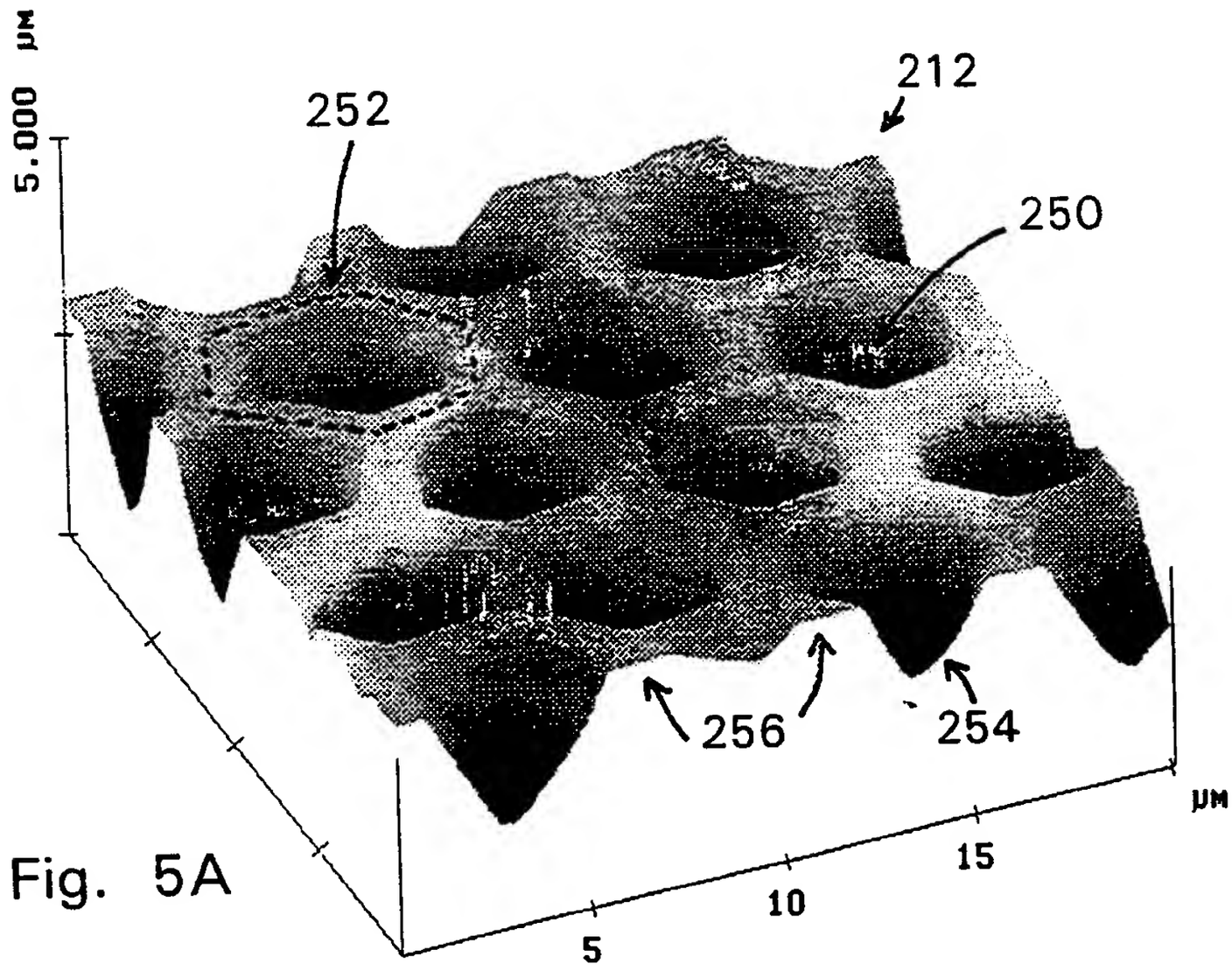

5.000 µM
252
212
250
256
254
µM
15
10
5

Fig. 5A

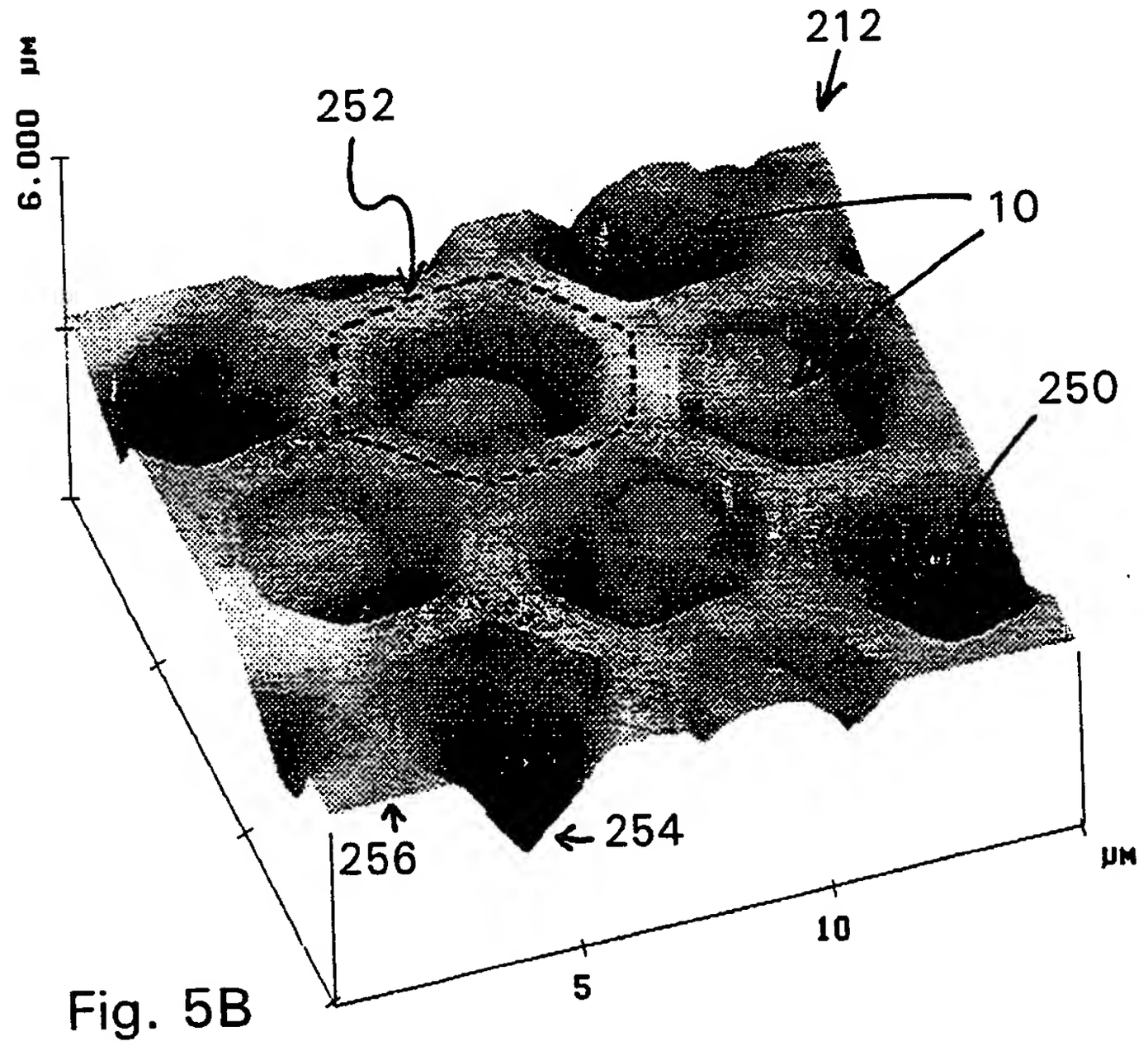

6.000 µM
212
252
10
250
256
254
µM
10
5

Fig. 5B

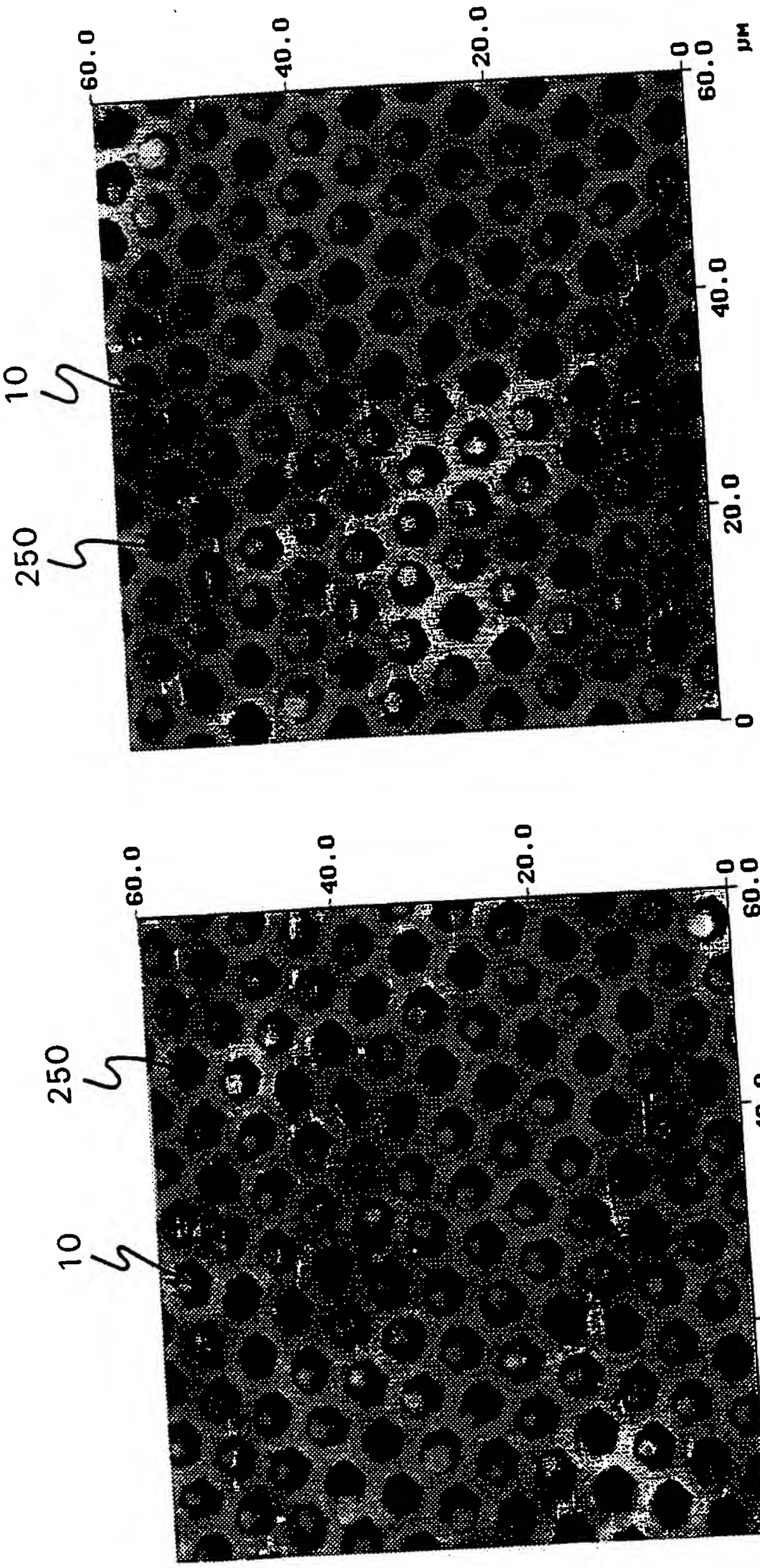

60.0
40.0
20.0
0
µm
60.0
40.0
20.0
0
10
250

Fig. 6B

60.0
40.0
20.0
0
µm
60.0
40.0
20.0
0
10
250

Fig. 6A

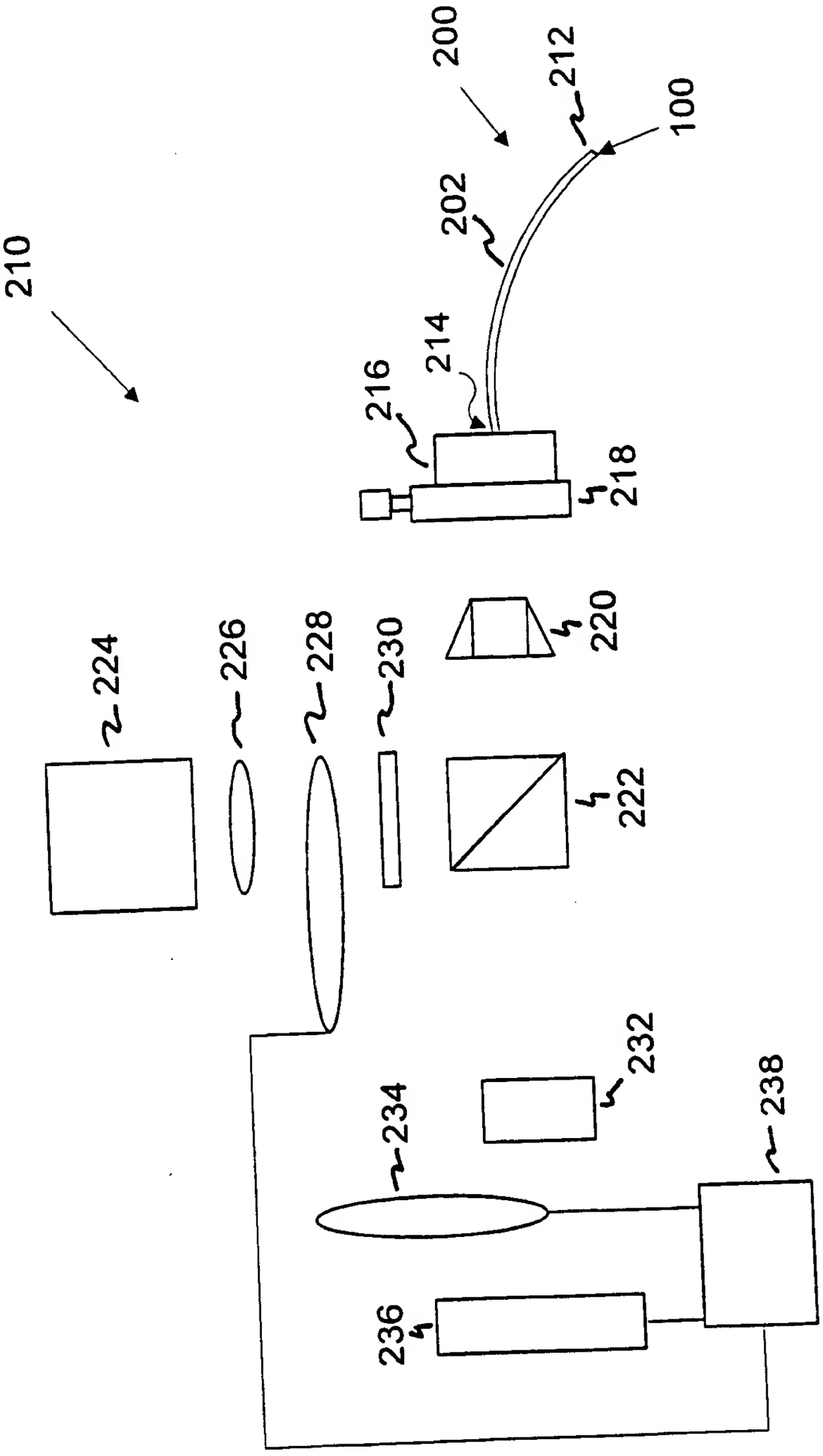

200
212
100
202
210
214
216
218
220
222
224
226
228
230
232
234
236
238

Fig. 7

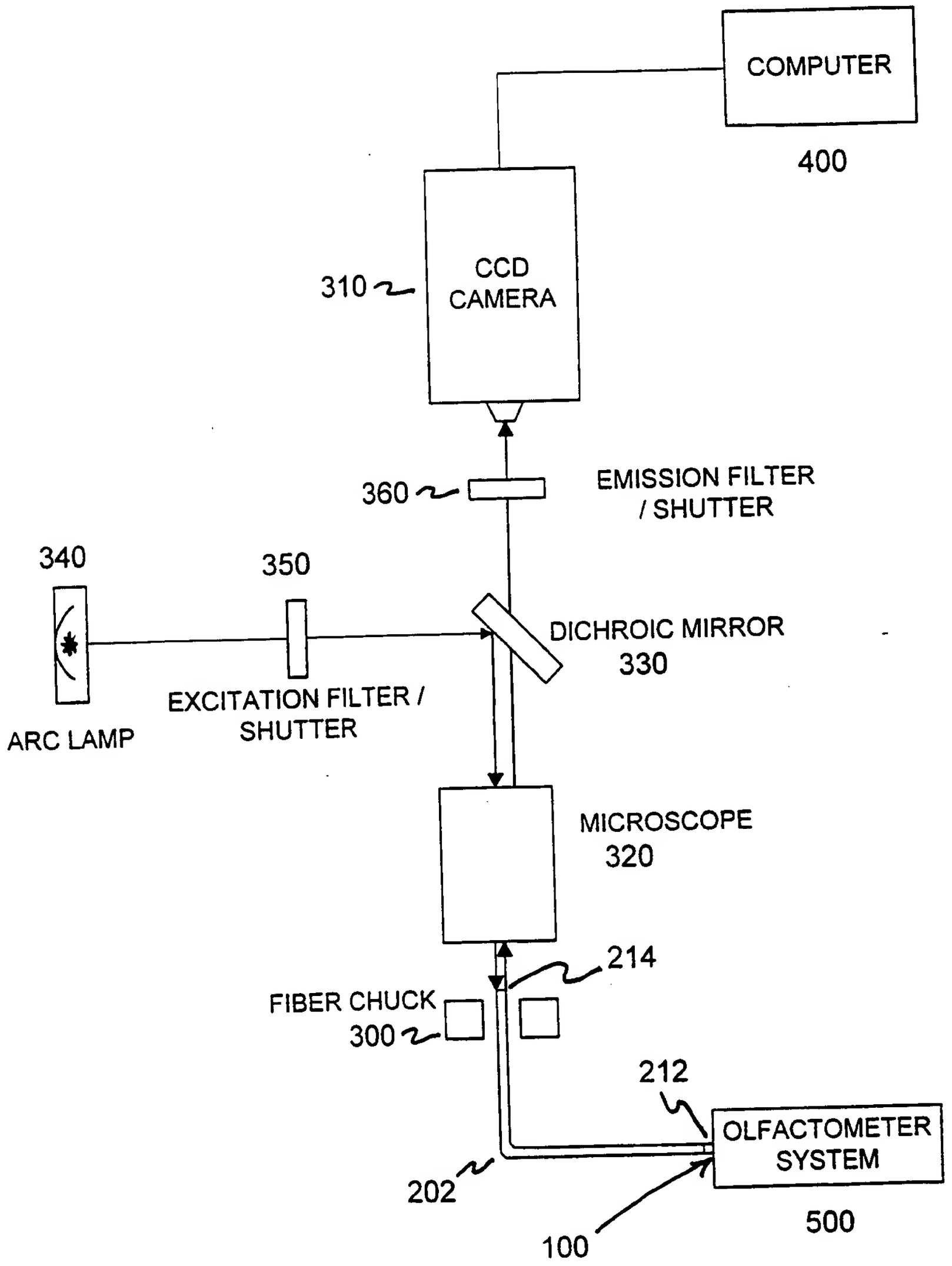

COMPUTER
400
CCD CAMERA
310
360
EMISSION FILTER / SHUTTER
340
350
DICHROIC MIRROR
330
ARC LAMP
EXCITATION FILTER / SHUTTER
MICROSCOPE
320
214
FIBER CHUCK
300
212
OLFACTOMETER SYSTEM
500
202
100

Fig. 8

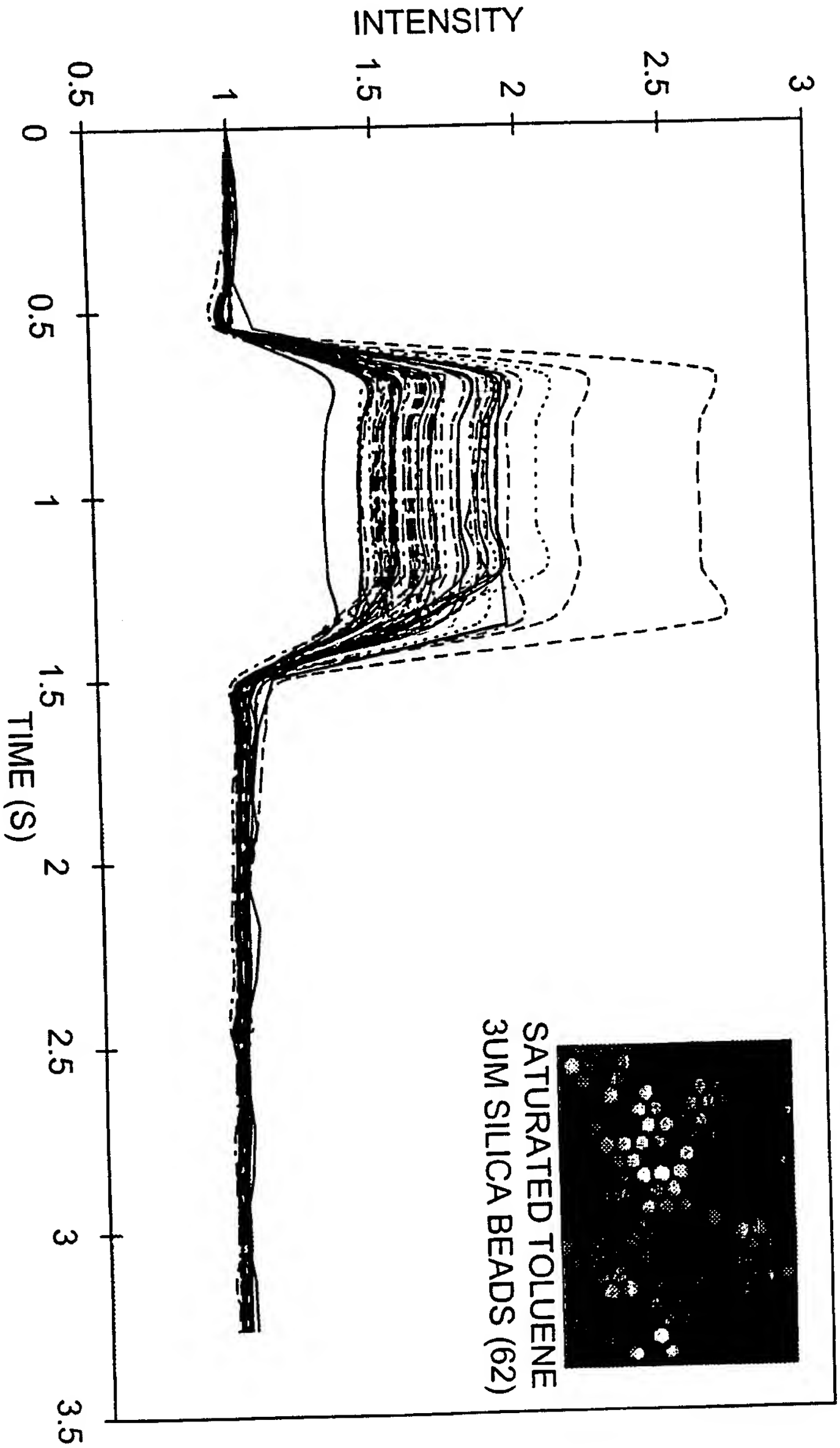

INTENSITY
0.5
1
1.5
2
2.5
3
0
0.5
1
1.5
2
2.5
3
3.5
TIME (S)
SATURATED TOLUENE
3UM SILICA BEADS (62)

Fig. 9

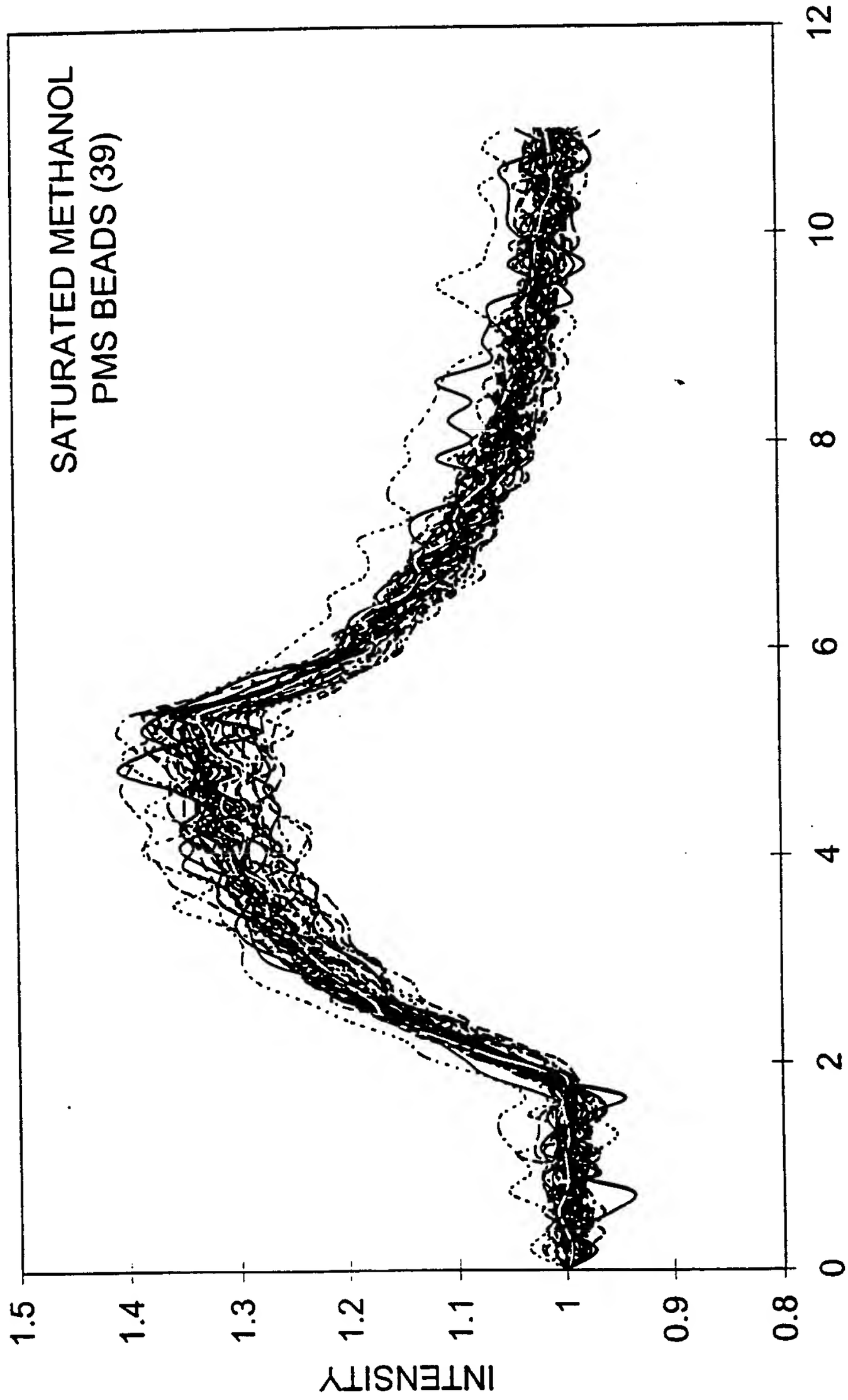

SATURATED METHANOL
PMS BEADS (39)
INTENSITY
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
TIME (S)
0
2
4
6
8
10
12

Fig. 10

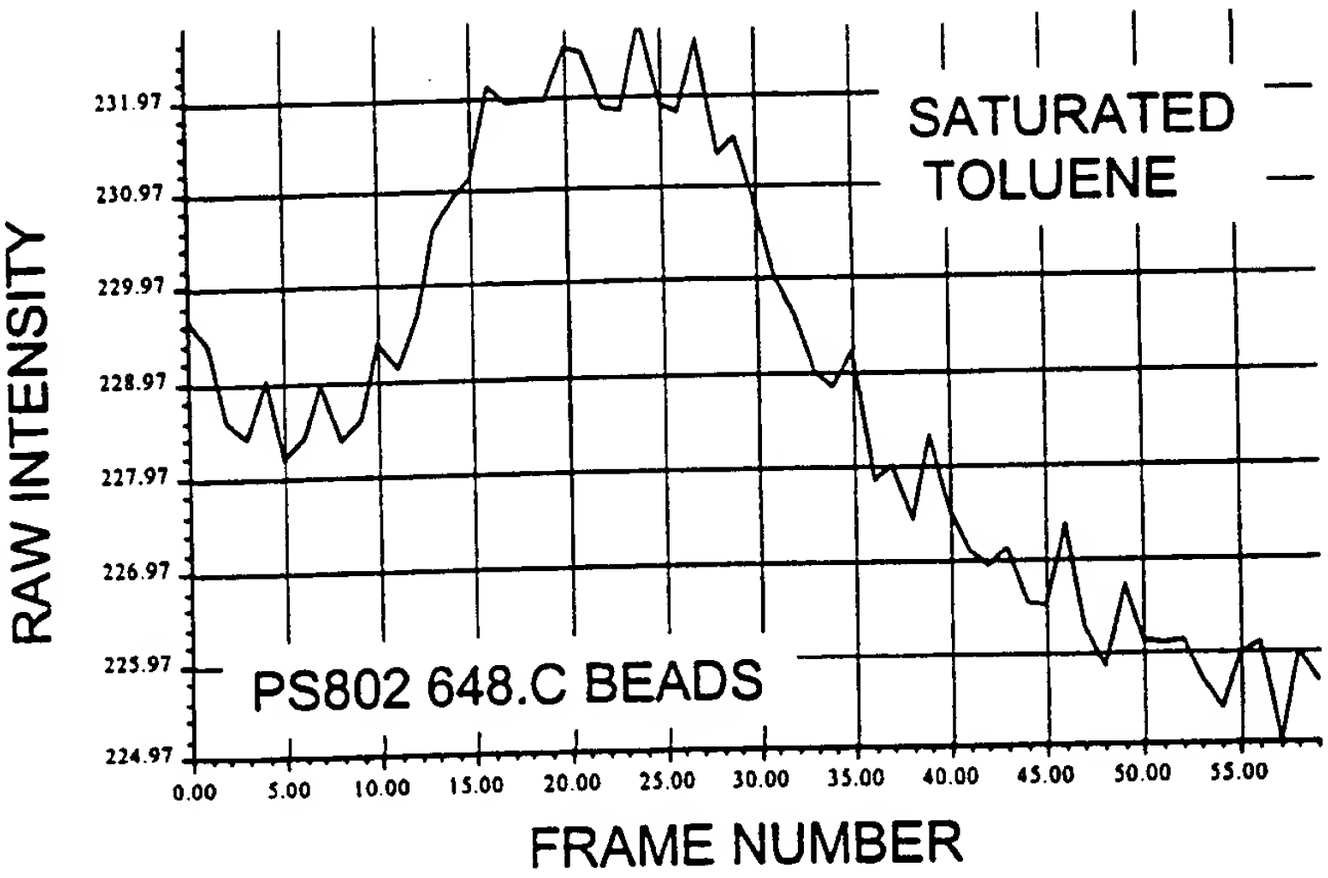


Fig. 11A

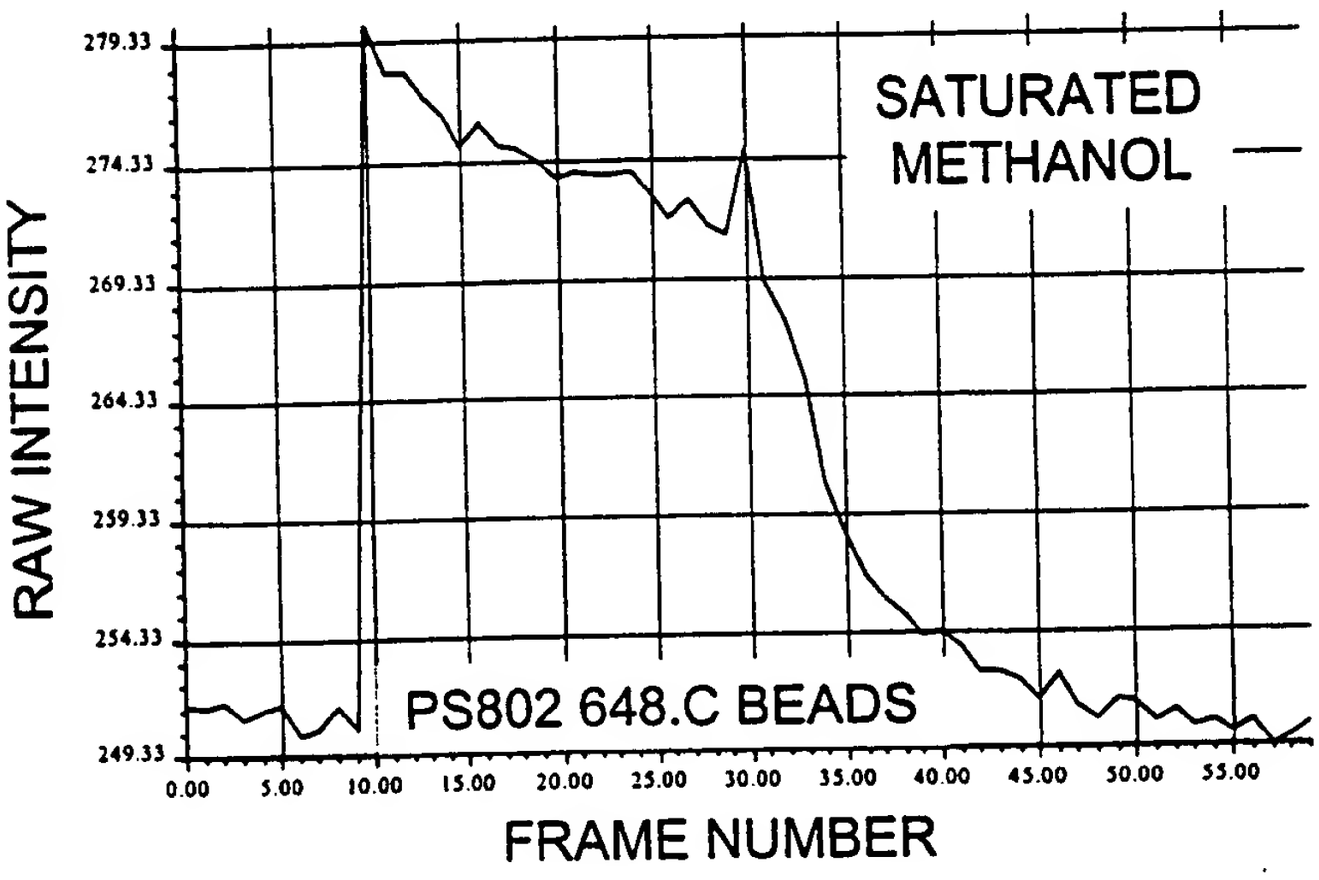


Fig. 11B

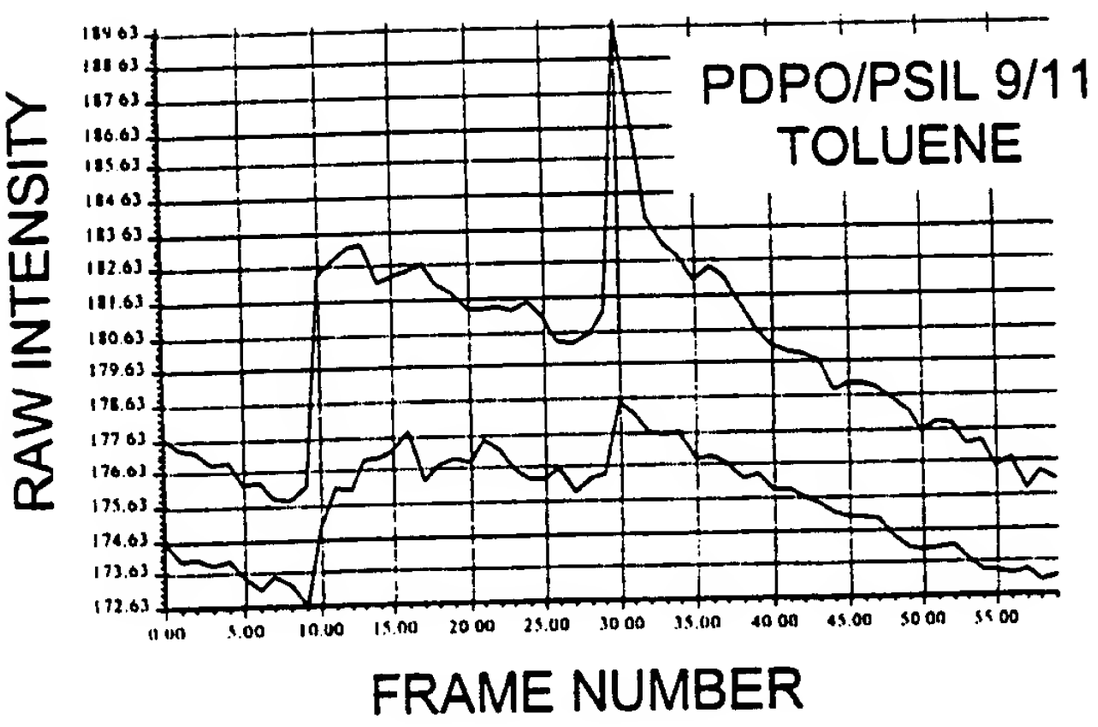


Fig. 12A

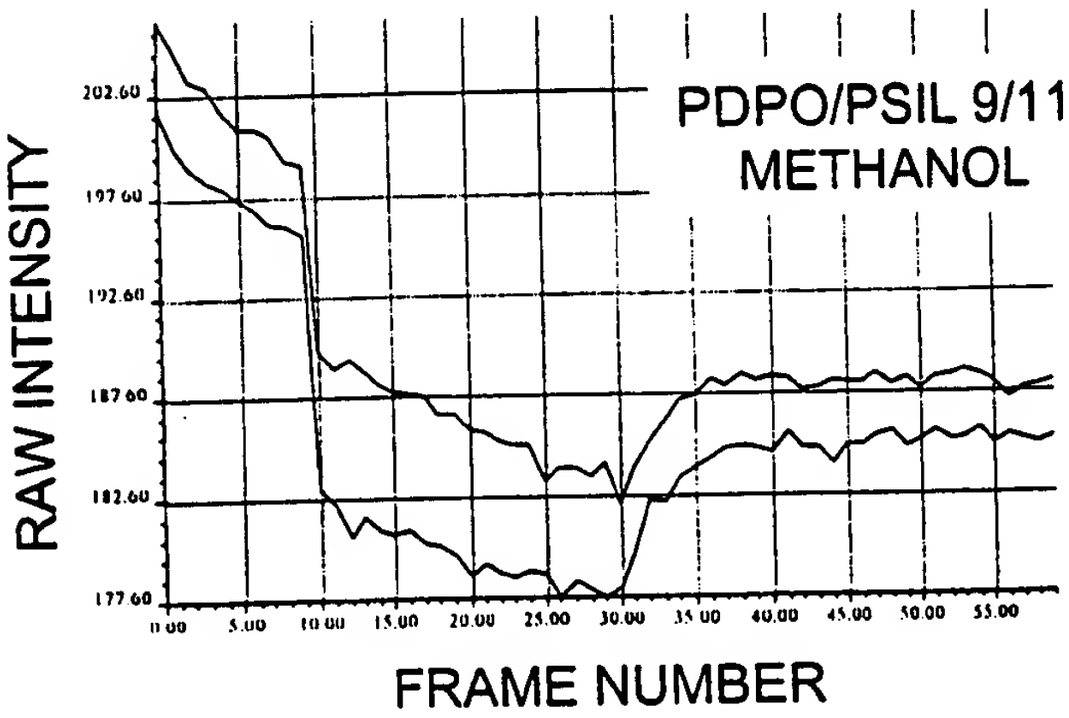


Fig. 12B

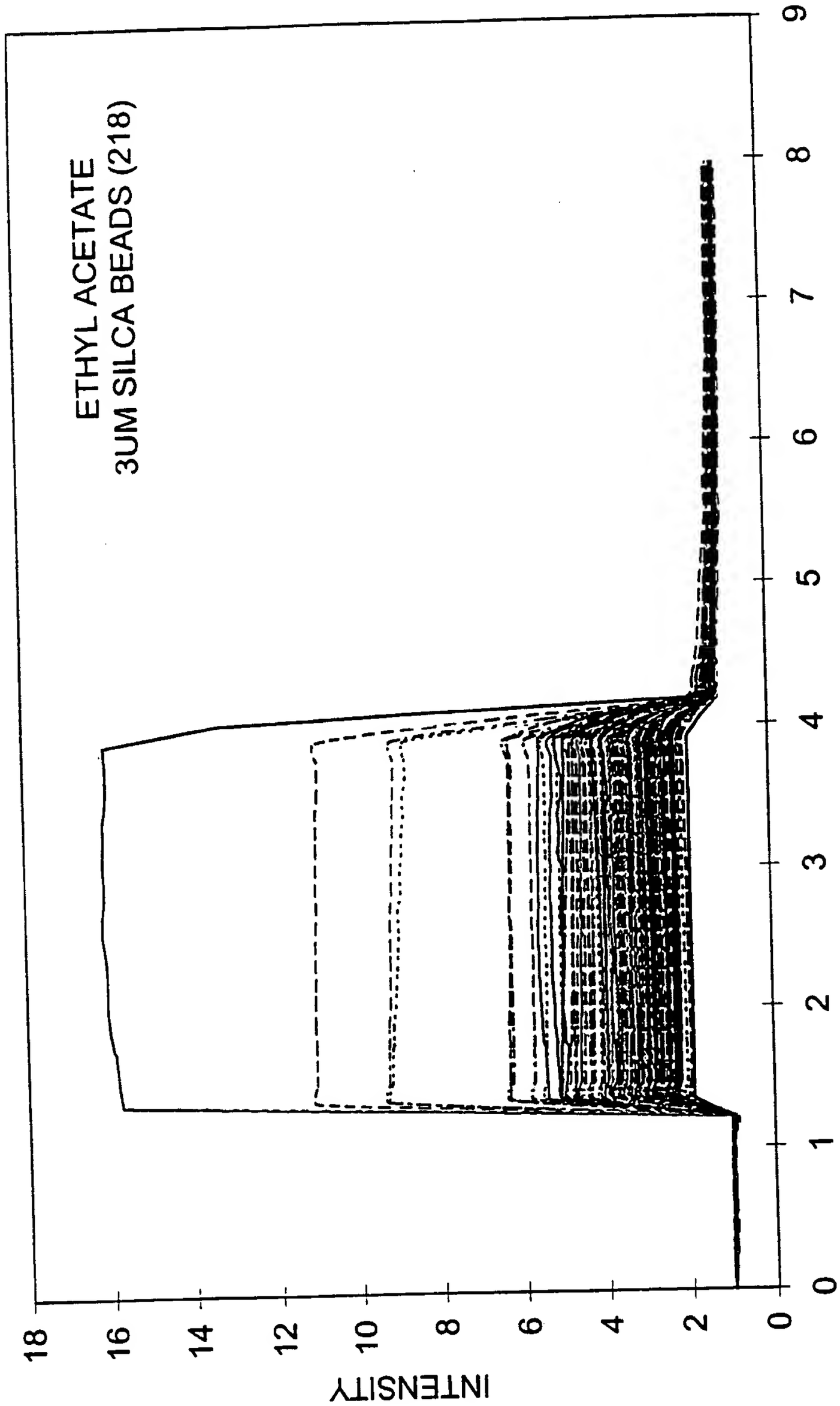

ETHYL ACETATE
3UM SILICA BEADS (218)
INTENSITY
18
16
14
12
10
8
6
4
2
0
TIME (S)
0
1
2
3
4
5
6
7
8
9

Fig. 13

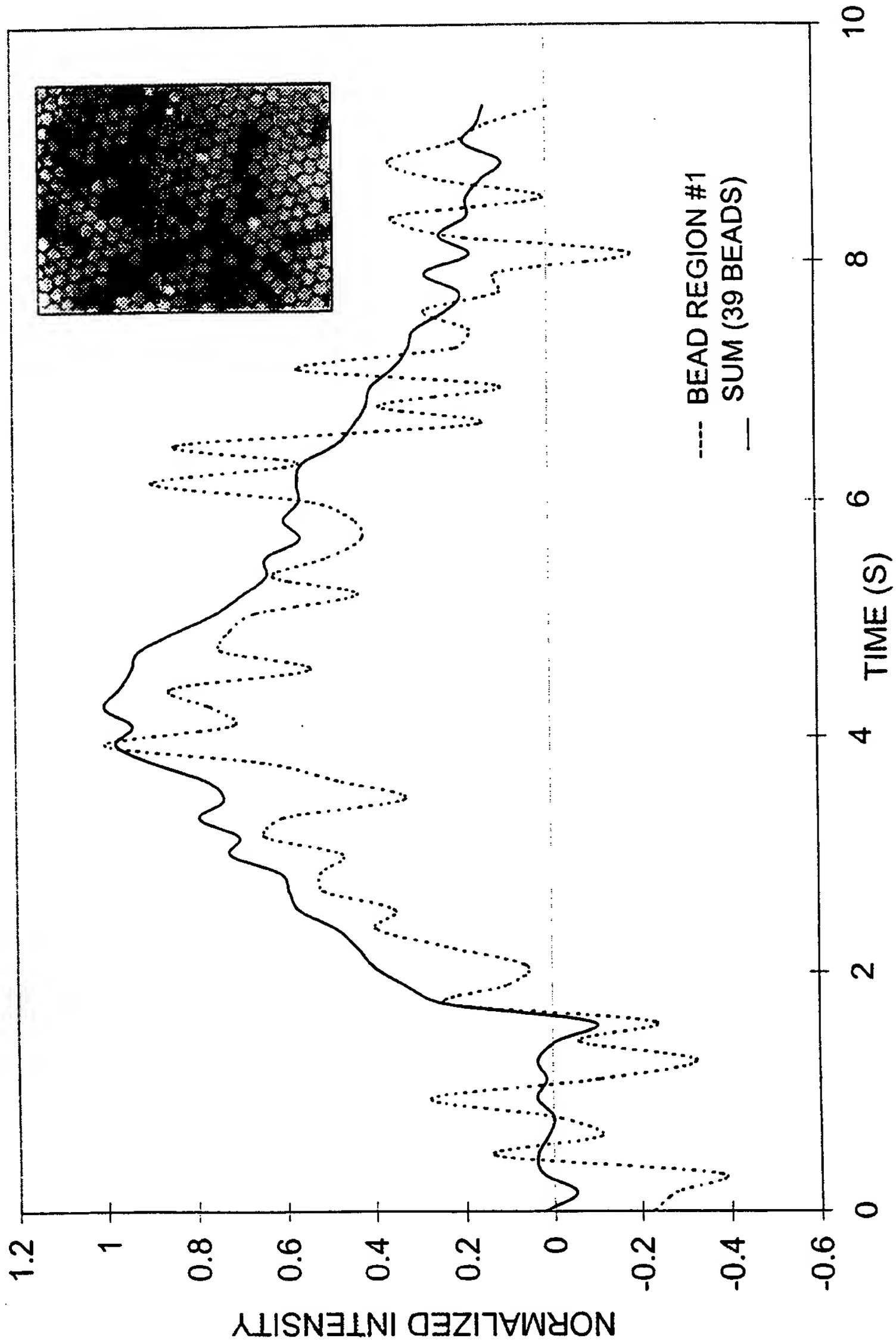

--- BEAD REGION #1
— SUM (39 BEADS)
NORMALIZED INTENSITY
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
0
2
4
6
8
10
TIME (S)

Fig. 14

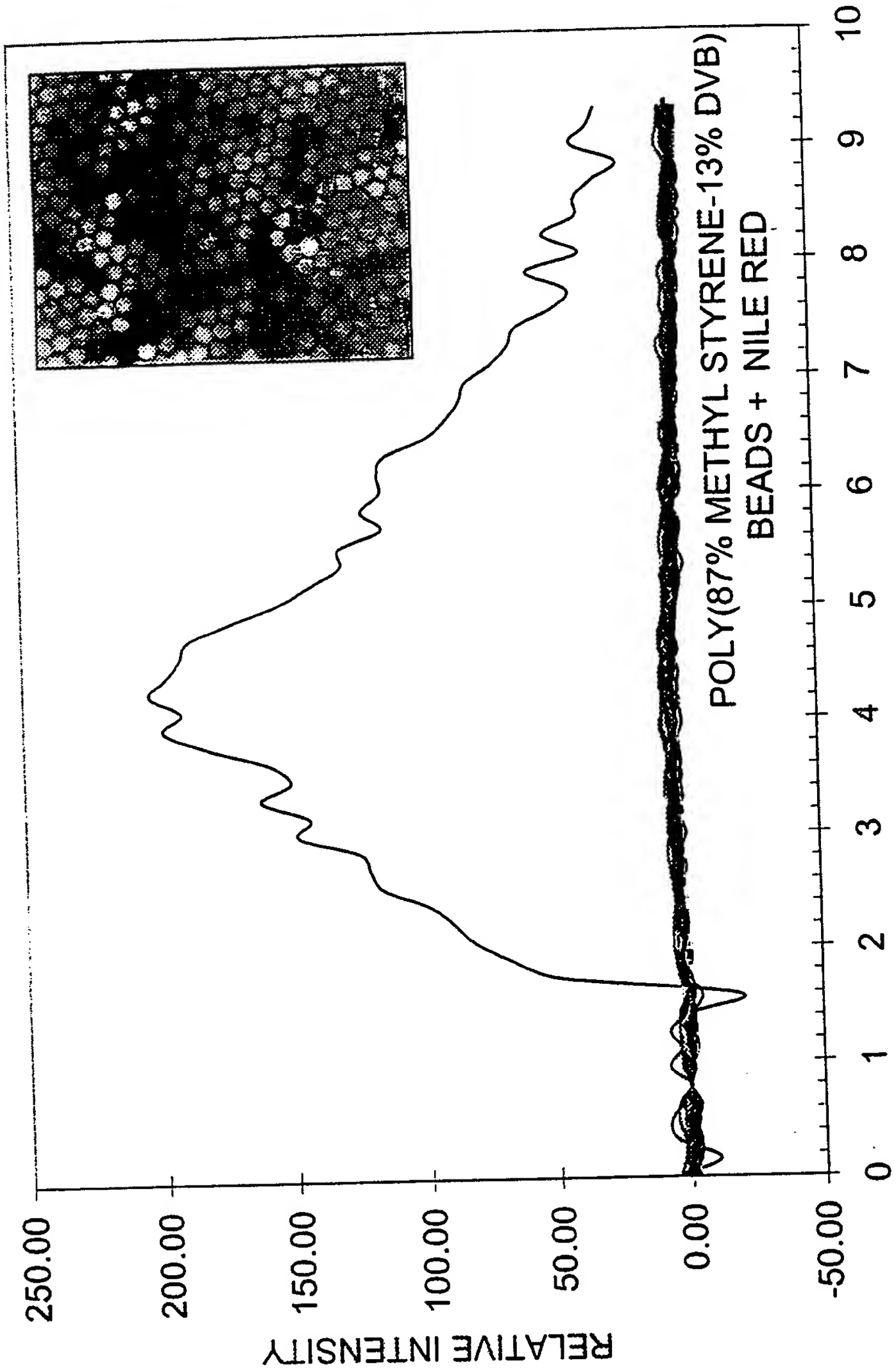

POLY(87% METHYL STYRENE-13% DVB)
BEADS + NILE RED
RELATIVE INTENSITY
250.00
200.00
150.00
100.00
50.00
0.00
-50.00
TIME (S)
0
1
2
3
4
5
6
7
8
9
10

Fig. 15

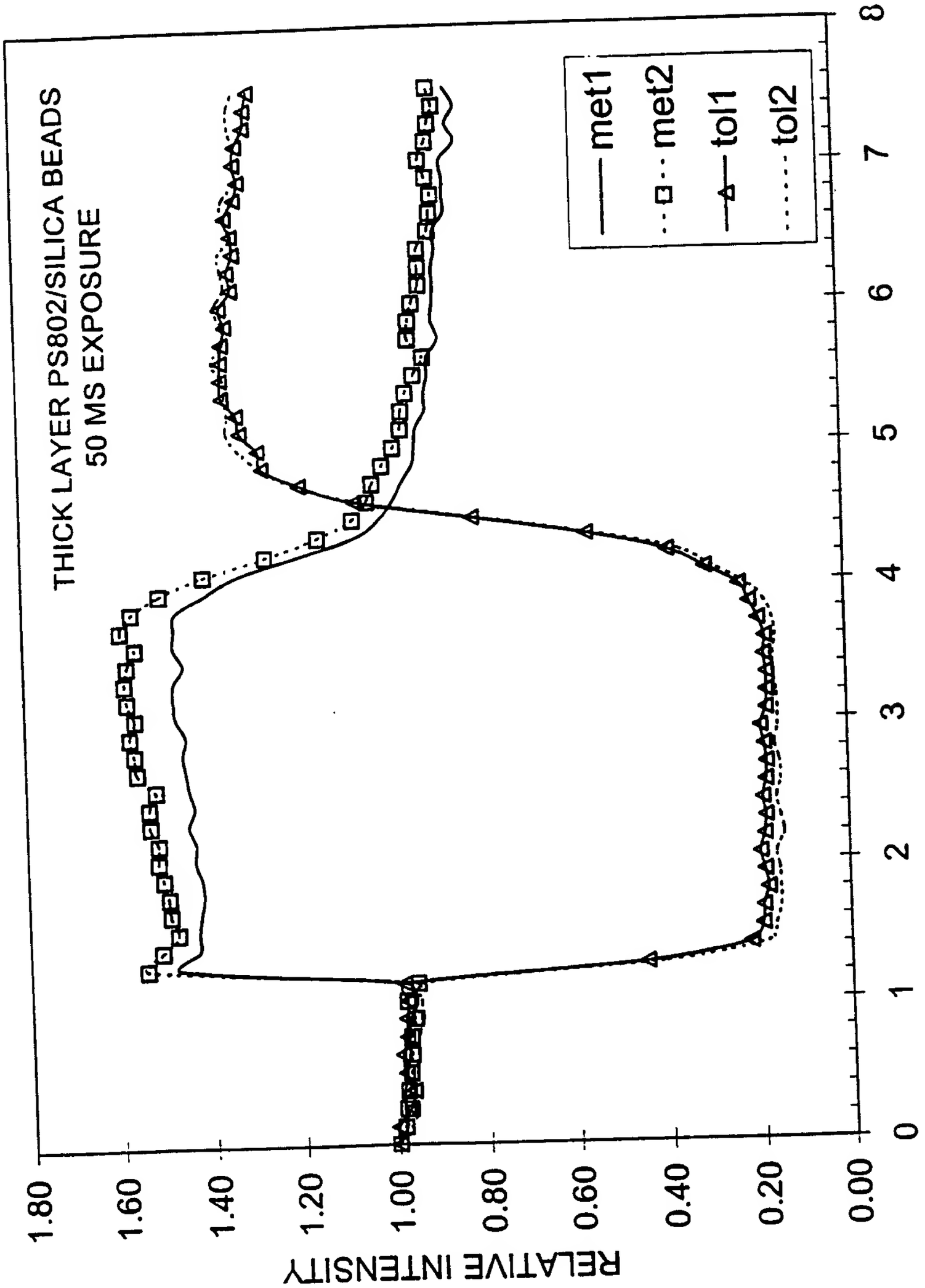

THICK LAYER PS802/SILICA BEADS
50 MS EXPOSURE
met1
met2
tol1
tol2
RELATIVE INTENSITY
TIME (S)
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0
1
2
3
4
5
6
7
8

Fig. 16

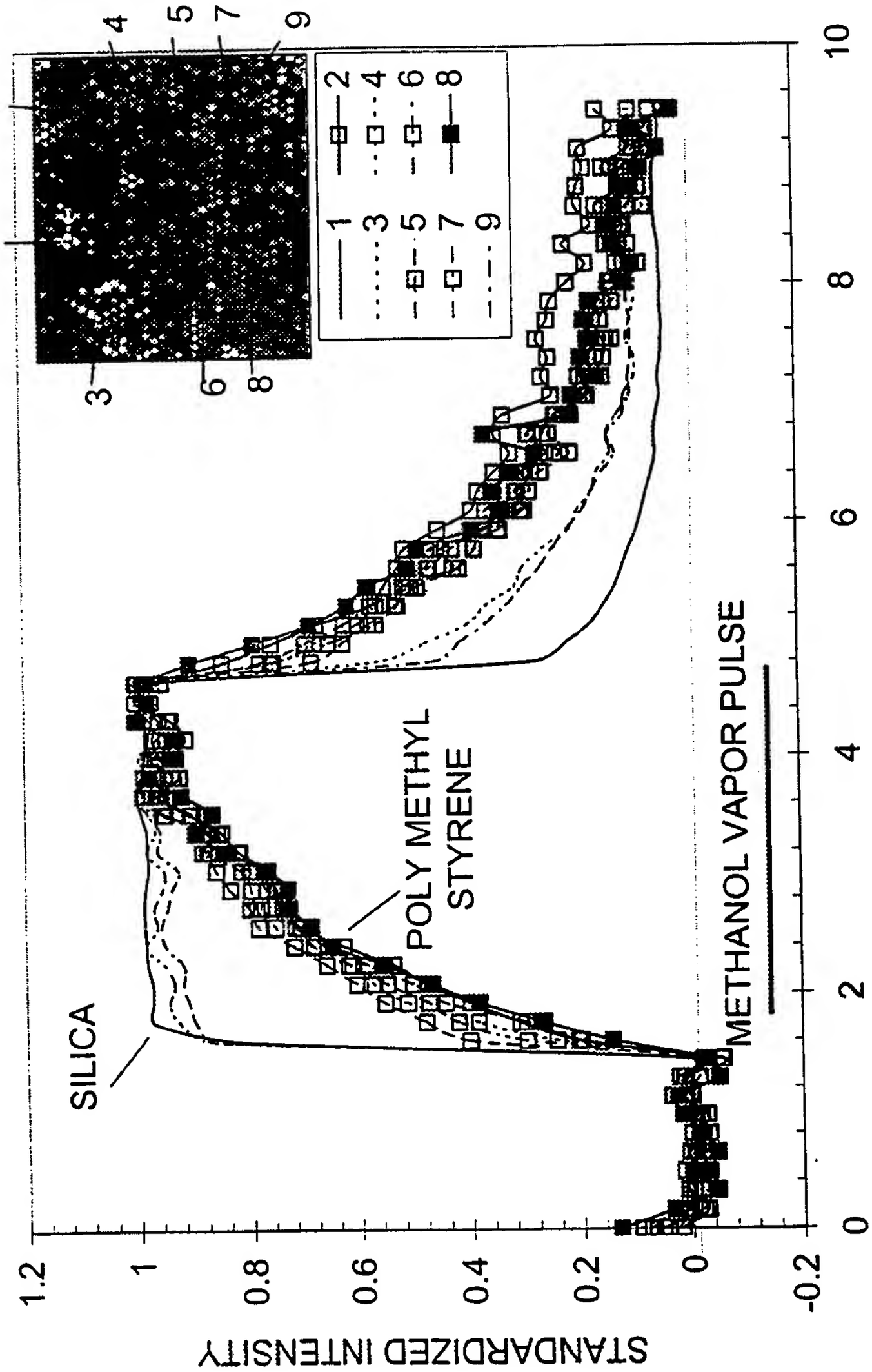

1
2
3
4
5
6
7
8
9
SILICA
POLY METHYL STYRENE
METHANOL VAPOR PULSE
STANDARDIZED INTENSITY
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
2
4
6
8
10
TIME (S)

Fig. 17

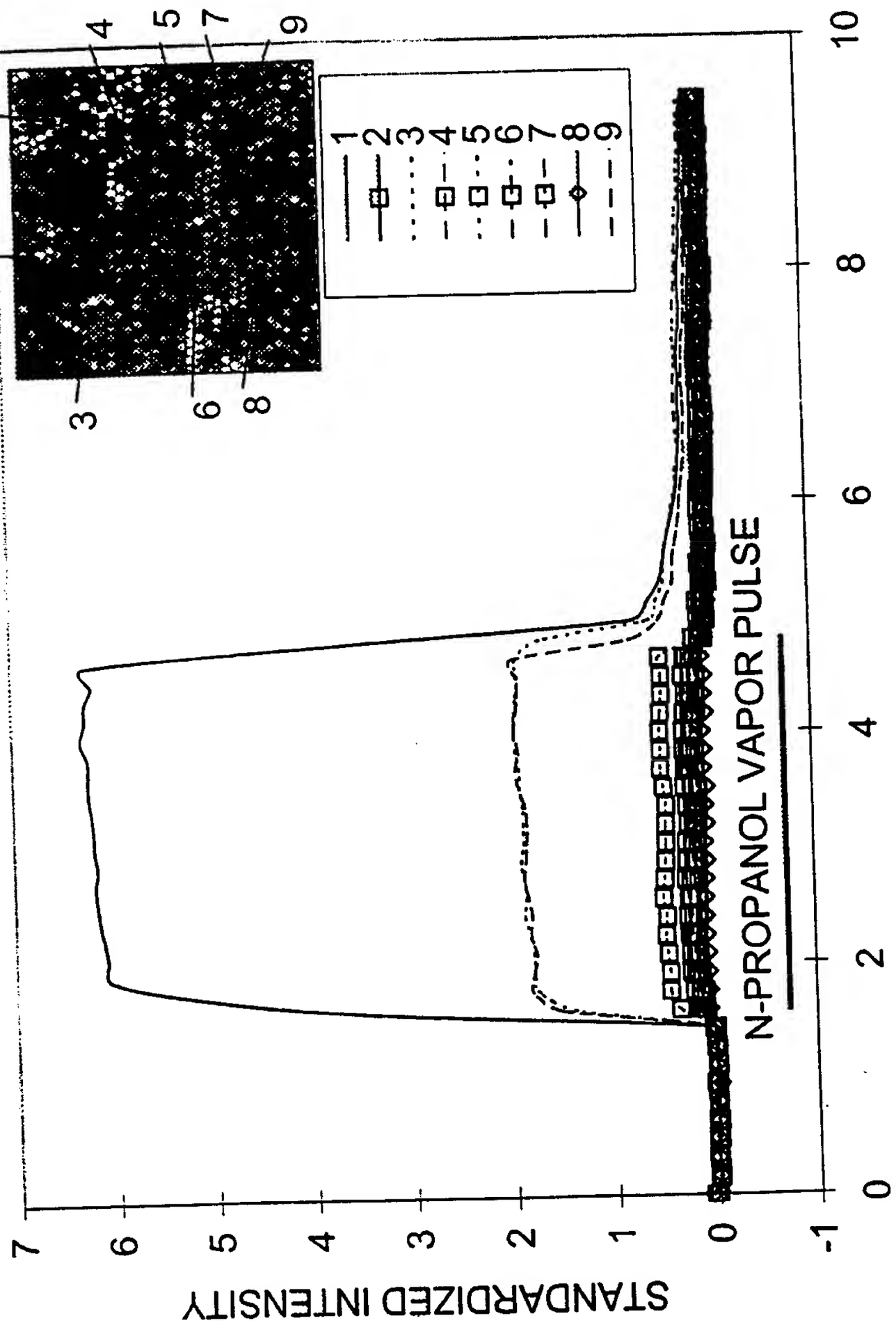

1
2
3
4
5
6
7
8
9
10
N-PROPANOL VAPOR PULSE
TIME (S)
STANDARDIZED INTENSITY
7
6
5
4
3
2
1
0
-1

Fig. 18

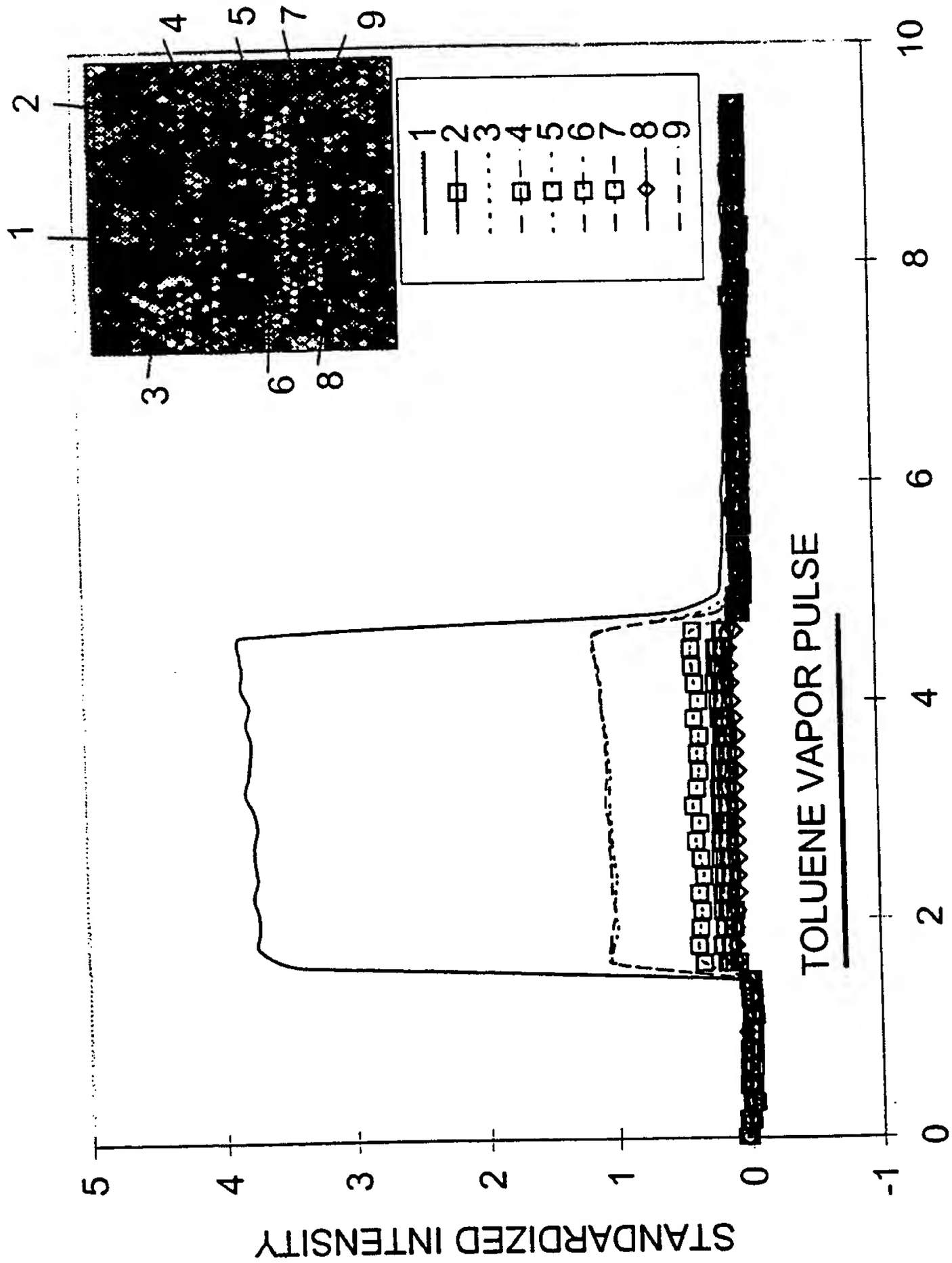
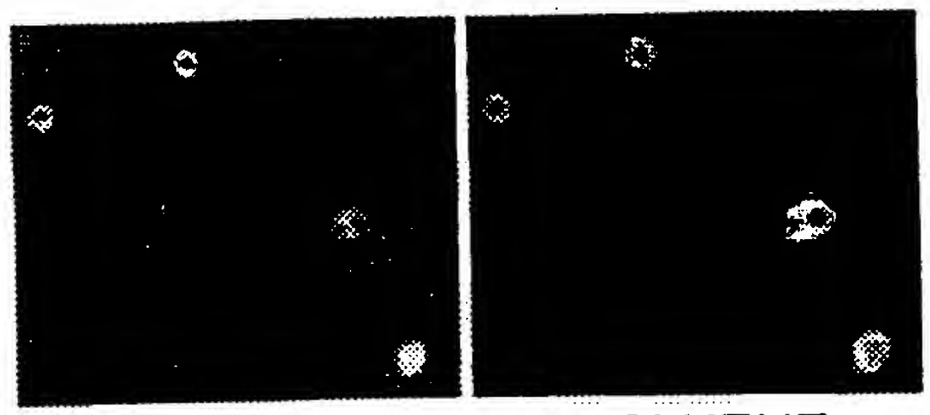

1
2
3
4
5
6
7
8
9
10
TOLUENE VAPOR PULSE
TIME (S)
STANDARDIZED INTENSITY
0
2
4
6
8
-1
0
1
2
3
4
5

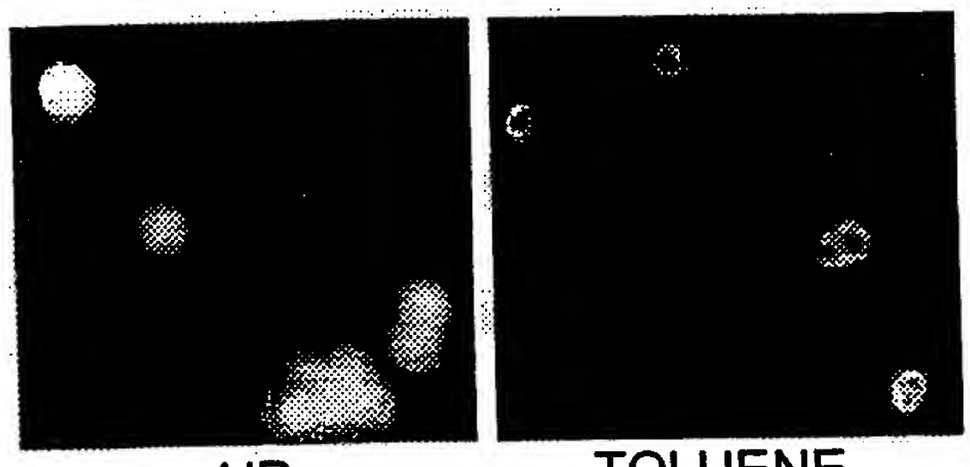
Fig. 19

PS802 648.C



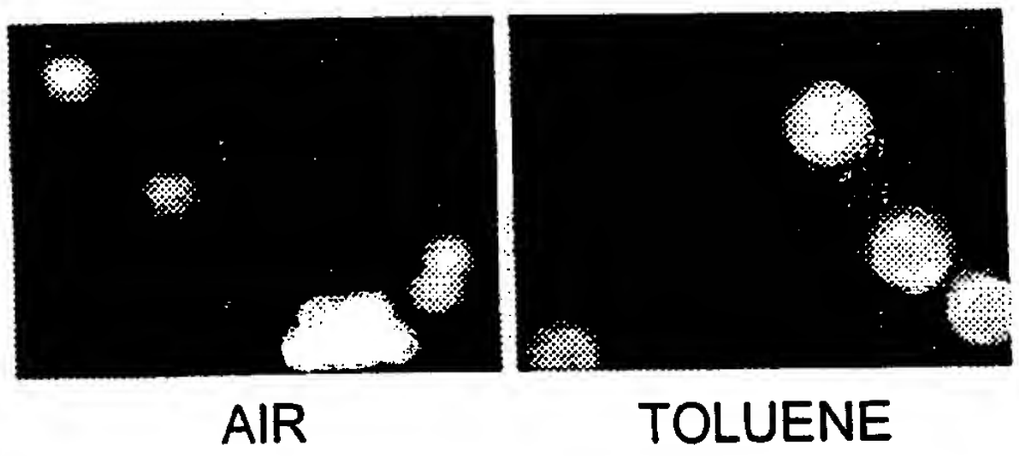
AIR TOLUENE

POLY METHYL STYRENE / 2% DIVINYL BENZENE



AIR TOLUENE

POLY METHYL STYRENE



AIR TOLUENE

Fig. 20

INTERNATIONAL SEARCH REPORT

nternational Application No
PCT/US 98/21193

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/50 G01N21/62 G01N21/77 G01N21/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 12863 A (TUFTS COLLEGE) 9 June 1994 see the whole document ---	1-42
A	US 4 499 052 A (FULWYLER MACK J) 12 February 1985 see the whole document ---	1,11,14, 16,33, 36,39,41
A	WO 97 12030 A (NANOGEN, INC.) 3 April 1997 see page 17, line 33 - page 53, line 26; figures ---	1,11,14, 16,33, 36,39,41
A	GB 2 294 319 A (CAMBRIDGE IMAGING LTD) 24 April 1996 see the whole document --- -/--	1,11,14, 16,33, 36,39,41

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 March 1999	31/03/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bosma, R

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/21193

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		JP 4047265 B	03-08-1992
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		JP 1035268 A	06-02-1989
		JP 2638085 B	06-08-1997
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		US 5254477 A	19-10-1993
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